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An in vitro study of
THE INFLUENCE OF SMEAR LAYER UPON
ROOT DENTINE PERMEABILITY
IN ENDODONTICALLY TREATED TEETH

This Thesis is submitted to the
University of Sydney in partial fulfilment
of the requirements for the degree of
Master of Dental Science - Endodontics

5th April 1989

David G H Cable BDS

STATEMENT OF AUTHORSHIP

This thesis is submitted as a partial requirement for the degree of Master of Dental Science (Endodontics), University of Sydney. Further requirements for the degree were previously completed within the Department of Operative Dentistry, University of Sydney, during 1985, 1986 and 1987.

This investigation was conducted within the Department of Operative Dentistry, at the United Dental Hospital of Sydney and Westmead Dental Clinical School, and with assistance from the Electron Microscope Unit of the University of Sydney.

No portion of this work has been submitted by the candidate to any other university for the award of any other degree or diploma, either in part or in full. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference is made within the thesis text.

A handwritten signature in black ink, appearing to read 'D G H Cable'. The signature is fluid and cursive, with the first letter 'D' being particularly large and stylized.

David G H Cable

5th April 1989

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ABSTRACT

Instrumentation of dentine has been shown to result in the production of a 'dentine smear layer.' This structure has received a great deal of attention within recent literature, generating considerable speculation regarding its desirability or potentially deleterious action. Techniques have been developed which are capable of removing the smear layer and have been strongly advocated by various proponents. However, the clinical significance of its removal from root dentine remains inconclusive.

The present study was designed to examine the influence of smear layer upon the diffusion of eugenol through intact root dentine, in vitro, within a model analogous to the clinical endodontic situation.

Smear layer was removed using the technique of Yamada et al (1983) involving sequential irrigation with 10 mls of 15% EDTAC solution followed by 10 mls of a 1% NaOCl (Miltons). Scanning electron microscopy was used to validate the effectiveness of this protocol in removing smear layer from instrumented root canals (N = 4 teeth).

Nineteen contra-lateral pairs of single rooted, homologous, human mandibular premolar teeth were used (N=38) in this study, which was conducted in three parts :

Part 1 consisted of the biomechanical preparation and subsequent obturation of teeth (N = 24), with a tritium (^3H)-labelled eugenol root canal sealer (Procosol) and laterally condensed gutta percha. Eluant samples were analysed by liquid scintillation spectrometry to measure the diffusion of tritiated eugenol (^3H -E), at logarithmically based 'end-time' intervals up to 100 days, both in the presence and absence of a dentine smear layer.

In Part 2 of the study, powdered samples of root dentine were prepared (N = 10 teeth) and analysed by liquid scintillation spectrometry to determine the mean eugenol concentrations in root dentine following the moment of peak outflow (T = 24 hours).

The rate release kinetics were also analysed for the influence of age, sex, post-extraction interval, irrigant volume, and the internal lateral surface area available for diffusion within each root canal.

The results indicated that smear layer, *per se*, did not significantly influence either the release kinetics or dentine concentration of eugenol within this intact root dentine model.

Peak eugenol outflow ($19.99 \text{ pmol.min}^{-1}$) occurred within 24 hours of obturation and was sustained, within one order of magnitude, for at least ten days post-obturation.

The eugenol concentration was found to range from a maximum of $0.23 \times 10^{-3} \text{ M}$, within the root canal, down to $5.22 \times 10^{-6} \text{ M}$ within peripheral root dentine. These concentrations suggest a possible anti-microbial action, within the root canal itself, but imply non-toxic effects upon the adjacent periodontal ligament tissues.

The results of this study suggest that removal of the smear layer, from endodontically instrumented teeth, in vivo, would be unlikely to potentiate the therapeutic and/or cytotoxic effects of eugenol released from a ZOE based root canal sealer. Therefore, it was hypothesized that smear layer removal was unlikely to detrimentally affect a tooth's prognosis when compared to conventional endodontic therapy without smear layer removal.

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CHAPTER 1

INTRODUCTION

It has long been held that if "the three basic principles of endodontic therapy are faithfully adhered to then the end result must be successful" (Weine, 1982).

These three principles, the "endodontic triad", are :

- a) thorough debridement of the root canal system,
- b) sterilization of its contents, and
- c) complete obturation of the space created.

Despite virtually unqualified acceptance of these principles, there have been numerous reports in the literature of apparent resolution of periapical radiolucencies without obturation (Dubrow, 1964; Barker & Lockett, 1969; Kennedy & Simpson, 1969; Barker, 1976; Moule & Oswald, 1983; Szajkis & Tagger, 1983; Lieberman & Towbridge, 1983). These case reports are conclusively supported by experimental data refuting Rickert & Dixon's "Hollow-Tube theory" of 1931 (Selye, 1959; Seltzer et al, 1963; Goldman & Pearson, 1965; Torneck, 1966, 1967; Hodosh et al, 1967; Davis et al, 1971; Wenger et al, 1978; Pittford, 1983).

Numerous investigators have also shown that we can not instrument root canals completely free of either organic debris, such as remnant pulp tissue and viable anaerobic bacteria, or inorganic debris which includes a dentine 'smear layer'.

Why then do we succeed at all, let alone with a 90⁺% success rate? (Kerekes & Tronstad, 1979; Ingle et al, 1985; Molven & Halse, 1988) Several hypotheses have been proposed to explain this enigma. One explanation, suggested by Torneck (1980), stated simply that "what we take out of a root canal may be more important than what we put back." His theory for 'success' is based upon an immunological understanding of the pathogenesis of periapical disease. Torneck suggested that removal of debris resulted in a reduction of the

'immunological loading' within host tissues, to a level which favours resolution rather than persistent chronic inflammation. Therefore, in his view, endodontic success and failure were dependent upon the individual balance between immunological loading and host immuno-competence rather than the idealized and unattainable principles of an "endodontic triad".

Contemporary literature has indirectly reflected this concept through the tendency of investigations to examine microscopic and ultra-structural qualitative differences between debridement techniques. Recently, attention has focused upon one specific element of root canal debridement, the dentine 'smear layer'. The term "smear layer" refers to that morphologically discrete surface layer of 'debris' produced iatrogenically following instrumentation of enamel, dentine, cementum and even calculus (vid. p. 20).

Goldman and co-workers (1981, 1982, Yamada et al, 1983) have shown that smear layer may be effectively and reliably removed from a root canal via a simple modification of current irrigation techniques. They proposed a large volume, post-instrumentation final flush (20 mls) with alternate solutions of 15% EDTAC (10 mls) followed by 10 mls of 5.25% sodium hypochlorite (NaOCl). Other authors have reported similar results using citric acid (Wayman et al, 1979; Baumgartner et al, 1984; Rocca & Duprez, 1985) and ultrasonics (Cunningham & Martin, 1982; Cameron, 1983; Cymerman et al, 1983; Haikel & Allemann, 1988).

A great deal of speculative discussion followed the detection and structure elucidation of the smear layer by Eick et al, (1970) and McComb & Smith, (1975). This discussion has principally concerned the clinical merits of smear retention or removal from within coronal restorative cavities and root canals. It has been a popular assumption that removal of the smear layer could only be advantageous. However, this has not been adequately established in other than an empirical or hypothetical sense, particularly with regard to the root canal.

It can not be assumed that simply because residual dentinal debris and smear layer may be demonstrated by scanning electron microscopy (SEM), that these features are necessarily detrimental or undesirable. Indeed, dentine chips are known to promote biologic closure of pulpal and apical wounds (Baume et al, 1971; Suzuki et al, 1973; Tronstad, 1978; Oswald & Friedman, 1980 and Safavi et al, 1985). Smear layer could function in a similar manner, providing a predominantly mineral covering over tubule openings, isolating remnant and possibly noxious substances to the root canal space (Lester & Boyde, 1977; Mjör, 1985).

The work, reported by Pashley and his co-workers in a series of over thirty papers, has conclusively shown that removal of the dentine smear layer potentially increases the permeability of coronal dentine between 4 – 32 times both in vivo and in vitro.

Therefore, it is possible that therapeutic substances placed within the root canal may be available in much greater concentrations at the periodontal root surface if the smear layer was removed.

Speculative opinions, such as these, should be considered within relevant clinical perspectives. Conventional endodontic therapy has achieved an impressive and predictable success rate prior to the recognition of, and our ability to remove, the dentine smear layer. Following the axiom, "first do no harm", any modification(s) to contemporary techniques and principles should only be adopted if it can be successfully argued that they would not decrease the current 90⁺% success rate. That is, alternatively, that such modification(s) should not exacerbate the existing potential for immunological, chemical or bacteriological pathogeneses within the root canal.

The purpose of this research was to investigate the effect of smear layer upon eugenol diffusion through intact root dentine, in vitro, within a model analogous to the clinical endodontic situation.

Eugenol, released from a zinc oxide-eugenol (ZOE) root canal sealer, was selected as the tracer molecule for this investigation since it is a fundamental component of many endodontic medicament and sealer formulations. Its therapeutic and toxic effects are known to be dependent upon eugenol availability, local tissue concentration, and the time for which the tissues are exposed to that concentration.

CHAPTER 2

REVIEW OF THE LITERATURE

- 2.1 Diffusion of Molecules Through Dentine
 - 2.2 Smear Layer
 - 2.3 Clinical Significance of Smear Layer Removal
 - 2.4 Eugenol and Zinc Oxide-Eugenol
-

2.1 Diffusion of Molecules Through Dentine

Dentine is a highly mineralized hard tissue which is composed of approximately 70% inorganic and 18% organic matter. The remaining 10-12% is present mainly as water within the dentinal tubules (Pashley et al, 1978a; Mjör, 1979). The mineralized inorganic fraction is primarily hydroxyapatite and the bulk of the organic components are collagen (Borggreven et al, 1979).

Despite their superficial appearances, enamel and dentine are not static impermeable tissues. Various dyes, molecules, and radio-isotopes have long been known to penetrate through enamel and dentine (Fish, 1927; Bodecker & Lefkowitz, 1938; Ross, 1941; Lefkowitz, 1943; Bartelstone et al, 1947; Amler, 1948; Wainwright & Lemoine, 1950; Kreudenstein, 1958; Marshall et al, 1960; Stevenson, 1965; Sorensen & Gatewood, 1966; Martin et al, 1968).

Numerous studies have qualitatively shown that fluid may move through enamel and dentine either outward from the pulp (Brännström, 1962; Berggren & Brännström, 1965; Anderson et al, 1967; Linden, 1968; and Johnson et al, 1973) or inward from the surrounding oral fluids (Langeland, 1957; Mjör & Tronstad, 1972; Bergenhöltz & Lindhe, 1975; Hume & Kenney, 1981; Dippel et al, 1984; Lindemann et al, 1985).

Several investigators have reported that hypertonic solutions applied to vital dentine would stimulate a painful response in both human and animal models (Anderson et al, 1958; Anderson &

Ronning 1962a,b; Anderson & Matthews 1967; Horiuchi & Matthews, 1973; Anderson & Matthews, 1976). It was suggested that the osmolarity of these solutions caused fluid to move through the dentinal tubules and that this fluid flux resulted in the neural stimulation of the pulp.

Brännström and co-workers proposed the hydrodynamic theory of dentinal sensitivity. They suggested that minute movements of fluid through dentine were responsible for the transduction of various clinically applied stimuli (thermal, osmotic, tactile and pneumatic) into dentinal pain (Brännström, 1960a,b & 1961; Brännström & Åström, 1964; Brännström et al, 1967,1968; Linden & Brännström, 1967; Brännström & Johnson, 1978). They concluded that histologic evidence of odontoblastic displacement was indicative of fluid movement through dentinal tubules in vitro (Brännström et al, 1967). Anderson et al (1967) also interpreted histologic signs as being indicative of fluid movement through dentine in response to the application of hyper-osmotic solutions in vitro.

One disadvantage of these dentinal sensitivity studies was that the stimuli applied were neither graded nor constant in magnitude or vector. Therefore, a direct relationship between response and stimulus could not be established (Horiuchi & Matthews, 1973).

Närhi (1978) and Närhi et al (1982) used positive and negative hydrostatic pressures in dogs and cats to overcome this limitation, but not without significant technical difficulty. They demonstrated, in vivo, that pulpal mechano-receptors would react to hydrostatic pressure gradients, irrespective of orientation. Their results supported the hydrodynamic theory of dentinal sensitivity but also indirectly showed that fluid could move through dentine in both directions.

Further analysis of the determinants of fluid movement across dentine required the development of techniques for the quantitative measurement of this movement within a consistent, reproducible model. Quantitative studies of dentine permeability have primarily

involved measurement of either radioactive solute diffusion or the measurement of solvent filtration (bulk fluid movement) across dentine. According to fundamental concepts of physiology and rheology, this movement should ideally conform to the following.

The rate of a solute's diffusion across a membrane is expressed by Fick's law¹ :

$$J = D_p \cdot A \cdot \frac{dC}{d\chi}$$

where J = solute flux, ($\text{cm}^2 \cdot \text{sec}^{-1}$)
 D_p = diffusion coefficient
 A = diffusional surface area, $\Sigma(\pi r^2)$ and r = tubular radius
 dC = difference in concentration across membrane
 $d\chi$ = thickness of the diffusion barrier

whereas filtration is calculated using the Poiseuille-Hagen equation² :

$$V = \frac{\pi \Delta P r^4}{8 \chi \eta}$$

where V = volume flow, ($\mu\text{l} \cdot \text{min}^{-1}$)
 ΔP = hydrostatic pressure gradient
 r = tubular radius
 η = viscosity
 χ = length of tube

The principle difference between these two methods of analysis is their dependence upon tubule radius ($J \propto r^2$ and $V \propto r^4$, respectively). Filtration is also dependent upon the viscosity of the fluid (η) whereas diffusion is independent of this factor. On the other hand, diffusion is dependent upon the solute concentration gradient (dc) whilst filtration is dependent upon solvent pressure gradient (ΔP).

Many problems have existed in the quantitative measurement of dentine permeability characteristics, primarily due to the significant geometrical complexities of even the most simple clinical model.

1 Best & Taylor : Physiological basis of Medical Practice; 11th Ed (1985)-Ch 6, p.129
 2 Skalak, R. & Chein, S.; Biorheology (1981); 18: 307-330.

Individual qualitative variations in the nature of dentine between specimens, and differences due to natural variations in both the external (e.g. enamel thickness, and uneven contour of the dentine-enamel junction) and internal (pulpal) anatomic contours, render strict quantification of data, within clinical models, impossible. Clinical cavity preparations were therefore abandoned in favour of conceptual models which permitted this standardization.

Outhwaite et al (1974) developed a conceptual model which was comprised of dentine discs mounted within a "Split-chamber" device. This apparatus, in turn, was positioned over a fraction or "eluant" collector. This model permitted strict quantification of an isotope's clearance from one chamber, through the intervening dentine, into a collection chamber and, therefore, calculation of its (isotope) rate of diffusion through the disc of dentine.

Each disc was cut from coronal dentine of a freshly extracted human third molar tooth. Each was of a known thickness and a standardized surface area, with each specimen serving as its own control for each variable or characteristic examined. This model permitted the simplified objective examination of the 'dependent' variable, under idealized and controlled conditions, without influence from extraneous or clinical factors.

One group of researchers, headed by D. H. Pashley, have used this model extensively to systematically and objectively investigate the prominent physiological factors which affect dentine permeability. Specifically, their first report (Outhwaite et al, 1976) determined that diffusion through dentine (D_p) depended most significantly upon the site/area from which each dentine disc was prepared. If all other factors were held equal then the more peripheral the location from which the dentine was prepared, the less its permeability. They concluded that peripheral or periodontal dentine was significantly less permeable than inner or pulpal dentine within the same tooth.

Outhwaite et al suggested this regional variation may be explained in simply anatomic terms by the relative dimensions of the dentinal structures and was consistent with predicted values derived using Fick's equation. They supported this suggestion with data from the work of Brännström and Garberoglio (1972) who showed that both the density and diameter of dentine tubules decrease as they extend peripherally towards the enamel surface in coronal dentine.

According to Fick's law, the diffusion of a solute across a membrane is directly proportional to the surface area of that membrane which is available for diffusion. Outhwaite et al proposed that the dentine discs of their model functioned as a barrier to diffusion according to the same equation. Hence, they concluded that peripheral dentine was less permeable due to its decreased tubule density per unit area and the decreased tubule diameter of this dentine, both of which resulted in reduction of the available "diffusional surface area".

Similarly, Pashley et al (1987) demonstrated both qualitatively (dye diffusion) and quantitatively (filtration), that there were distinct and consistent differences in the regional distribution of fluid movements through coronal dentine. They reported that the patterns of permeability were similar for both the intact crown segments and the dentine discs, even though only the discs had uniform thickness. They concluded that the non-uniform permeability pattern in discs of standard thickness indicated inherent regional differences in dentine permeability which could not be explained solely by either dentine thickness or tubule length. They suggested that anatomical variations in the structure and orientation of dentinal tubules, which could be directly attributed to dentinogenesis, were responsible for these "regional permeability variations" throughout every tooth.

Numerous anatomic and microscopic studies have reported that quite significant anatomical changes occur in the morphological characteristics of dentine depending upon the site from which each sample/specimen was prepared (Brännström & Garberoglio, 1972;

Garberoglio & Brännström, 1976; Whittaker & Kneale, 1979; Baume, 1980; Tidmarsh, 1980; Thomas & Carella, 1983; Thomas & Payne, 1983; Carrigan et al, 1984 and Thomas & Carella, 1984).

Recent studies on regional variability of dentine suggest that the permeability of radicular dentine is significantly less than that of coronal dentine (Fogel & Marshall, 1987; Krell & Zeni, 1987; Fogel et al, 1988). However, these studies used longitudinally sectioned dentine 'slabs' prepared from lateral root surfaces, and differences in methodology which do not allow direct comparison against earlier data from Outhwaite's coronal dentine model.

Pashley (1988) stated that radicular dentine was about 10^{-1} to 10^{-2} times less permeable than mean coronal dentine permeability, measured by filtration, but unfortunately did not present his methodology for critique.

Outhwaite et al (1976) also demonstrated that doubling the surface area doubled the rate of radioactive iodine (^{125}I) diffusion. Similarly, increasing the temperature from 25°C to 35°C and decreasing the dentine thickness of a sample would increase the rate of ^{125}I permeation. Pashley et al (1983) also demonstrated that increasing the temperature $+40^{\circ}\text{C}$ would increase hydraulic conductance (filtration) between 1.8 and 4-fold.

In 1977, Pashley et al, studied in vitro the rate of diffusion across human dentine of four water soluble radioactive molecules i.e. : tritiated water ($^3\text{H}_2\text{O}$); pertechnetate ($^{99\text{m}}\text{TcO}_4^-$); iodine ($^{131}\text{I}^-$) and urea (^{14}C -urea). The experiment was conducted in three parts to examine : the influence of pulp tissue, remaining dentine thickness and the comparative rates of permeation for each molecule.

The four molecules studied were of similar ionic radii, ranging from $1.97\text{\AA}$ for water to $2.70\text{\AA}$ for urea. Water and urea were selected as examples of uncharged molecules whilst both iodine and pertechnetate were used as examples of (negatively) charged molecules.

Pashley found that the rates of 'permeation' (diffusion) were proportional to their ionic radii, i.e. $D_p : {}^3\text{H}_2\text{O} > {}^{131}\text{I}^- > {}^{99\text{m}}\text{TcO}_4^- > {}^{14}\text{C}\text{-urea}$. They concluded that dentine diffusion varied inversely with solute size (ionic radius) and the latter was more important than either charge or molecular weight in determining the rate of diffusion for any given molecule. They also showed an inverse relationship between remaining dentine thickness and rate of diffusion consistent with the results of Outhwaite et al (1976). The extirpation of pulp tissue had only a minor effect upon diffusion kinetics whilst application of a cavity varnish (Copalite) resulted in a 69% reduction in ${}^{131}\text{I}$ permeation.

Merchant et al (1977) compared the diffusional permeation of radioactive iodine (${}^{125}\text{I}$) with its bulk fluid flow (i.e. filtration) both before and after acid etching of dentine. The rate of ${}^{125}\text{I}$ filtration, following application of a hydrostatic pressure equal to 240 cm of water (176 mm of Hg), was double the resting rate due to diffusion before acid etching. A profound 32-fold increase in ${}^{125}\text{I}$ filtration was found after acid-etching for 2 minutes with 50% citric acid. However, acid etching both sides of the dentine disc did not significantly increase the equilibrium rate of ${}^{125}\text{I}$ diffusion. They concluded that acid-etching, by removing the occluding surface debris (smear layer), facilitates filtration more than diffusion.

Reeder et al (1978) confirmed the dramatic 32-fold increase in the rate of filtration across dentine following the removal of surface debris by acid-etching. They also described a more refined model for the measurement of filtration or hydraulic conductance (L_p) across dentine.

Pashley & Livingston (1978) examined the effect of increasing molecular size (radius) on the rate of dentine diffusion for ten (10) substances ranging in size from 1.97 Å to 37 Å. A 19-fold increase in molecular radius resulted in a 100-fold decrease in diffusion across "polished" or smeared dentine, supporting their earlier work. However, two molecules (chlorhexidine and fluoride) diffused

significantly less than their calculated 'size predicted results' indicating that these molecules may become bound to dentine.

In contradiction to Merchant et al (1977), this study determined that acid-etching significantly increased dentine permeability, a 4-fold increase for water (1.97Å) and a 9-fold increase for iodine labeled albumin (37Å). They speculated, that the proportionately greater increase in albumin versus water permeability, indicated that surface debris (smear layer) imposes a greater restriction to the diffusion of large molecules relative to small molecules. Thus, if dentine thickness and physical surface area are held constant, then the "nature" of the dentine surface may be an important determinant of dentine permeability. SEM techniques confirmed the removal of surface debris (smear layer) after acid-etching, similar to the findings of Brännström & Johnson (1974).

This concept of the "nature of the dentine surface", spawned several investigations. The first, by Michelich et al (1978), examined the difference between 'anatomically' and 'functionally' determined tubular radii. The results of this study showed that 'functional' tubule radii (0.04 to 0.19 µm), calculated from Poiseuille's equation of filtration, range in effective size from 5 to 40% of their respective 'anatomic' radii (0.45 to 0.75 µm), measured using SEM techniques. They also demonstrated that acid-etched dentine had 'functional' tubular radii which were 1.8 to 4.3 times greater than the same specimen of polished (smeared) dentine.

The second, by Pashley et al (1978b), introduced the concept of dentine as a "diffusional barrier composed of a relatively impermeable mineralized hard tissue matrix traversed by numerous, uniform cylindrical water-filled tubes". They expanded upon this concept to explain their previous conclusions, based on the concepts that substances ideally would permeate through the 'water filled tubule' component of dentine according to the equations of Fick and Poiseuille-Hagen.

They proposed that the data of this study supported these conclusions and specifically, that the rate at which a substance permeates this barrier was proportional to its effective dentine diffusional surface area ($SA = A \cdot \rho$), i.e. the product of the available dentine tubule surface area (A), and the anatomic tubule density (ρ). Diffusion (D_p) was shown to increase proportional to dentine tubule surface area ($A = \pi \cdot r^2$), or to the square of the tubule radius (r^2), whilst inversely proportional to dentine thickness (χ). On the other hand, filtration (L_p) increased proportional to the square of the dentine surface area (A^2), or to the fourth power of the tubule radius (r^4), and decreased inversely with increasing dentine thickness (χ).

In this investigation, Pashley et al (1978b) used three methods i.e., SEM, isotope diffusion and gravimetric/volumetric measurements, to calculate mean tubular surface areas per unit area of dentine. They determined that the mean "diffusional surface area" of coronal dentine disc specimens was 10.2% to 10.5%. However, they also calculated that the overall range of "fractional" tubule surface areas, in coronal dentine, could vary from 1% of the dentine surface at the dentino-enamel junction to 22% at the dentino-pulpal junction respectively. This compared quite favourably with the purely 'optically' measured results of Garberoglio & Brännström (1976) who found that tubule volume varied from 4% at the dentine-enamel junction (DEJ) to 28% at the pulpal surface.

Pashley et al (1978a) explored this concept and substantiated the importance of diffusional surface area through an analysis of the resistance to fluid movement (filtration) through dentine. They described the total resistance across dentine as the sum of three potential resistances placed in series; i.e. surface resistance, tubular resistance and pulpal resistance. "Surface resistance", due to the presence of debris occluding the dentinal tubule orifices, accounted for 86% of the total resistance whilst internal irregularities within the tubules and pulpal remnants had little effect. Surface resistance was measured before and after acid-etching for 2 minutes with 50% citric acid to remove the 'surface debris' (smear layer).

Pashley et al (1979) and Pashley & Whitford (1980) discussed dentine as a true semi-permeable membrane, i.e., permeable to solvent but not to solute. They measured the ratio between the effective osmotic pressure of various substances across dentine and their theoretical osmotic pressures. This ratio was termed the "reflection coefficient". The magnitude of this reflection coefficient may range from zero (0.0), when a membrane makes no discrimination between solute and solvent, to 1.0 when only water (the solvent carrier of these solutions) was permitted to permeate.

Pashley et al (1979) showed that the reflection coefficient would vary inversely with the size of the solute molecule. Furthermore, they reported that there appeared to be a 'critical' molecular size (less than 15Å) below which the reflection coefficient would remain relatively unchanged despite altering the nature of the dentine surface through the removal of surface debris by acid etching or its (smear layer) analogous replacement through potassium oxalate treatment.

Recently, van der Graaf & Bosch (1988) devised a theoretical mathematical model which described the dependence of hydraulic conductance upon the observed decrease in tubule radius and tubule density in a pulp to enamel direction. This model substantiated the significance of "functional tubular radii" as suggested by Michelich et al (1978), however, its authors concluded that, theoretically, the influence of etching, and presumably smear layer removal, upon tubular radii and hence hydraulic conductance would be slight.

Pashley & Whitford (1980) also calculated the reflection coefficient(s) of various salts & sugars in dentine and established that the effective osmotic gradient induced by these small molecules was about 10^{-4} of their theoretical osmotic pressures. These data indicated that dentine does not discriminate well between water and low molecular weight solutes with small molecular radii such as glucose, urea and sodium chloride.

Pashley (1979) and Pashley et al (1981a) described an in vivo model which permitted quantitative determination and comparison of the permeability characteristics of dog dentine both in vivo and in vitro in the same teeth. They used ^{131}I diffusion to demonstrate that the rate(s) of permeation were similar both in vivo and in vitro.

The effects of dentine thickness, surface area and tubule patency (smear layer) were predictable and consistent with earlier observations made in vitro. They therefore concluded that if permeability increased in vivo as dentine thickness decreased, this indicated that the rate-limiting step across dentine was diffusion and not pulpal blood flow or clearance. The perfusion rate of the pulp chamber was varied 10 fold, from 0.1 to 1.0 ml/min, without effect. Removal of the pulp tissue following in vivo permeability measurements did not significantly affect the diffusion of ^{131}I .

Pashley et al (1983a) also examined the effects of acid-etching on filtration across dog dentine, in vivo. The results again indicated that the rate limiting step was filtration across dentine, rather than removal of the fluid by the pulpal circulation. However, the resultant 5-fold increase in filtration, in vivo, was dramatically lower than the previously reported 32-fold increase found in human dentine, in vitro (Merchant et al, 1977; Reeder et al, 1978).

Other in vivo studies have also demonstrated metabolic exchanges between vital pulp tissue and the oral cavity through methods using the systemic and topical administration of enzymatic and isotopic tracers (Wennberg & Bawden, 1978a,b; Tanaka, 1980; White et al, 1980; Potts et al, 1985 & Bergenhöltz et al, 1987). These investigations added further support to the in vitro model data of Pashley's group, correlating these with data from the clinical situation.

As the major independent physiological factors affecting dentine permeability were progressively reported, researchers began to specifically identify the effects of 'clinical' variables. One of the earlier factors known to influence permeability was the presence or absence of debris (e.g. smear layer) on the cut dentine surface.

Surface phenomena, and specifically the smear layer, have been shown to greatly affect fluid movement through dentine by both filtration (Reeder et al, 1978; Boyer & Svare, 1981; Pashley et al, 1981c; 1983a) and, to varying degrees, also by diffusion (Merchant et al, 1977; Pashley & Livingston, 1978; Pashley et al, 1978b; Boyer & Svare, 1981; Dippel et al, 1984 and Pashley & Galloway, 1985a).

Michelich et al (1980) reported that filtration of a bacterial suspension across dentine, with an intact smear layer, resulted in the virtual sterilization of the saline medium. However, they also demonstrated that bacteria could be forced, or filtered by pressure, through dentine if the smear layer was removed by acid-etching. They suggested that bacteria lodged within the tubules and smear layer thereby further decreasing the number and diameter of tubule orifices available for fluid movement. They concluded that bacteria could effectively reduce the "diffusional surface area" of dentine and, therefore, the ability of fluids to permeate through it.

Similarly, Pashley et al (1981f) used hydrostatic pressures, comparable to intra-pulpal pressures, to force washed red blood cells suspended in saline into dentinal tubules, in vitro. They demonstrated a significant 17-fold decrease in filtration across previously acid-etched dentine discs. The histologic appearance of erythrocytes forced into dentine, in vitro, was very similar to that found in dog pulps after crown preparation without adequate cooling, in vivo. Therefore, they concluded that the in vitro and in vivo processes were similar.

Pashley et al (1982) reported that saliva, bacterial suspensions and plasma proteins would significantly decrease filtration across dentine discs in vitro. Other studies supported and were able to correlate the influence of similar clinical variables through in vivo data derived from dogs (Pashley et al, 1981e; 1983b; 1984a; 1984b and Potts et al, 1985).

Pashley et al (1983b; 1984b) demonstrated a progressive, time related, decrease in dentine permeability following cavity preparation in vivo. They directly related this finding to the presence of vital pulp tissue and the extravasation of plasma proteins. Furthermore, Pashley et al (1984a) and Potts et al (1985) demonstrated a profound reduction in dentine permeability due to the effect(s) of large plasma proteins such as fibrinogen and α -aminoisobutyric acid.

Pashley et al (1985) concluded that air-evaporation could not explain this progressive reduction in dentine permeability, in vitro, but they attributed this reduction to the possible effects of another plasma protein, albumin.

Recently, several investigators have also used these quantitative methods to assess the influence of various clinical agents on dentine permeability. Greenhill & Pashley (1981) attempted to explain the effects of 28 clinically used desensitizing agents by correlating the changes produced in hydraulic conductance (L_p), or filtration, with observed topographic alterations in the dentine surface when viewed under the SEM.

Veaco et al (1982) reported that ^3H -eugenol could diffuse through human coronal dentine, in vitro, and that the concentration of the eluant was of the order 10^{-5}M eugenol within one hour. Following this, Hume (1984a) determined the rate of diffusion and the concentration gradient established across human dentine by the release of ^3H -eugenol from ZOE temporary restorations placed into freshly extracted human third molar teeth. ^3H -eugenol was released at rates which declined exponentially with time and were directly

proportional to the liquid-powder ratio but not the absolute mass of the mixture(s). The intervening dentine was shown to have a profound effect upon both the rate and degree of eugenol released, decreasing its peak efflux a 1000-fold.

Similarly, Pashley et al (1985, 1986 & 1988), Hume (1987) and Reynolds et al (1987) have investigated the effects of cavity liners, varnishes and bases on human dentine permeability, in vitro.

Abbott et al (1988) studied the diffusion of both the steroid and antibiotic components of Ledermix paste from the root canal to the periodontal surface of human tooth roots. They showed that the rate limiting step was diffusion through the lateral root dentine and not through the apical foramen, as might be expected. Furthermore, they confirmed that the total amount of tracer released was directly related to the area of contact between paste and dentine and not the paste volume, *per se*. In general, these results were consistent with the conclusions of Outhwaite et al (1976), Pashley et al (1978b) and Hume (1984).

Recently, Wang & Hume (1988) investigated the diffusion of hydrogen (H^+) and hydroxyl (OH^-) ions from various agents through dentine with quite unexpected results. They showed that H^+ ions from strong acids diffused through dentine poorly relative to that from either weak acids or tritiated-water. OH^- ions also diffused less readily than water. They speculated that the poor penetration of these ions was very probably due to the buffering capacity of components within dentine, such as hydroxyapatite.

The results of the above study were in direct contrast with those which would be predicted in light of previous data (Pashley et al, 1977; Pashley & Livingston, 1978; Pashley et al, 1979a) indicating that molecular size was a prime determinant of dentine permeability. However, Wang & Hume's data provides an explanation for earlier qualitative observations regarding the lack of acid diffusion through dentine (Marshall et al, 1960; Cohen et al, 1970; Fraser & Laws, 1976), in contrast to the clinically apparent pulpal toxicity of weak acids.

This data may also help to explain the reported interaction(s) of various molecules with dentine (Rotberg & Shazer, 1966; Pashley & Livingston, 1978; Meryon & Jakeman, 1987; Ciarlone et al, 1987).

It is clearly evident from the preceding literature that various molecules and substances are capable of moving through the ostensibly impenetrable hard tissues of teeth via mechanisms of both diffusion and filtration.

Furthermore, these studies have also identified several specific and fundamental physiological factors which significantly influence the rate and degree of dentine permeability. The most important of these factors, with regard to dentine diffusion, are :

- dentine diffusional surface area (tubule diameter $\times$ density)
- dentine thickness,
- regional and topographic characteristics of the dentine surface,
- specimen temperature during investigation, and
- the nature of the diffusing solute molecule or ion (i.e. size, concentration, and water or lipid solubility), and its binding affinity or interaction with the components of dentine.

Conversely, several of these studies have indicated that permeability remains relatively stable as a function of post-extraction time (Outhwaite et al, 1976; Pashley et al, 1981a; 1984b) and that neither the presence nor absence of pulp tissue appear to have a significant affect upon permeability kinetics, either in vitro (Pashley et al, 1977; 1978a; 1981a; 1981b) or in vivo (Pashley et al, 1981a; 1983b; 1984b).

2.2 Smear Layer

The terms "smear layer", "dentine smeared layer" and "smeared dentine" have been used interchangeably throughout the literature to refer to a morphologically discrete layer of 'debris' produced iatrogenically following instrument manipulation of enamel, dentine, cementum and even calculus. However, within this thesis, the term "smear layer" has been used for consistency.

Early attempts to define the cut surface of tooth structure were limited by the resolving power and specimen preparation techniques of light microscopy (Street, 1953; Peyton & Mortell, 1956; Lammie, 1957). It was not until the introduction of the SEM that Boyde et al (1963) first referred to the grinding debris created during cavity preparation as having been "smeared".

Eick et al (1970) identified and defined this layer of debris as a definitive structure. They described the smear layer, in coronal cavity preparations, to be "composed of an organic film less than 0.5 μm thick into which particles of apatite, ranging from 0.5 - 15 μm , were imbedded". They reported that smear layers were observed on all cut tooth surfaces but they were not necessarily continuous or homogeneous.

Jones et al (1972) found that smear layers were common on surfaces of cementum and calculus, as well as enamel and dentine, following the use of periodontal instruments.

Numerous contemporary studies have also reported the presence of a 'dentine' smear layer on all the instrumented root canal surfaces of endodontically treated teeth (Baker et al, 1975; McComb & Smith, 1975; McComb et al, 1976; Moodnik et al, 1976; Lester & Boyde, 1977; Goldberg & Spielberg, 1982; Goldman et al, 1981, 1982; and Mader et al, 1984).

The topographical morphology of smear layers has been extensively examined using the SEM. It has been stated to vary widely depending upon various factors, such as : whether the tooth was cut

dry or wet; the type of instrument used; the amount and chemical composition of the irrigating solution; and the size and shape of the restorative cavity or root canal (Brännström et al, 1979a; 1980; Gilboe et al, 1980; Pashley et al, 1981c; Bowen et al, 1984; Gwinnett, 1984; Berry et al, 1987). However, the ultra-structural composition of the smear layer has proved to be below even the resolution of standard SEM techniques (Pashley et al, 1987).

Eick et al (1970) described the smear layer produced on cut tooth surfaces, *in vivo*, as an organic film less than 0.5 μm thick into which 0.5 - 15 μm particles of apatite were imbedded. Based on mapped electron microprobe analyses, they concluded that the smear layer was primarily an organic film. Unfortunately, the resolution of this technique was not sufficiently refined to allow discrimination between the smear layer *per se* and possible incidental contaminants.

Conversely, Lester & Boyde (1977), using SEM, deduced that the smear layer produced within the root canal was essentially composed of "translocated mineralized tissues" since rendering the specimen anorganic, by prolonged digestion in 5% NaOCl, did not significantly alter the morphology of this layer. They did, however, also report the co-incidental inclusion of pulp debris and pre-dentine within the smear layer if the specimen was not previously digested.

Several other authors have speculated, either intuitively or from SEM topographic observations, that smear layers must be composed of heterogeneous mixtures of both organic and inorganic fractions (McComb & Smith, 1975; Moodnik et al, 1976; Cameron, 1983; Gwinnett, 1984; Mader et al, 1984).

Goldman et al (1981), using stereoscopic SEM photomicrographs determined that the thickness of the smear layer in instrumented root canals was about 1 μm but they were unable to assess the depth of penetration into the underlying dentinal tubules. They also showed that a smear layer was only present in those areas of the canal which had been instrumented.

Brännström (1982) reported that the thickness of clinically produced smear layers was approximately 1-5 μm in coronal restorative cavities. Mader et al (1984) described it as being 1-2 μm thick in the root canal but penetrating up to 40 μm into each dentinal tubule. They too speculated that the smear layer consisted primarily of fine particles of calcified tissue.

Bowen et al (1984) investigated the smear layer using both SEM and TEM techniques. They showed that the thickness of this layer varied widely but was of the order of 0.0 - 3.0 μm deep. They also observed denaturation of intertubular collagen in the subjacent region, to a depth of about 1.0 μm , and the inclusion of amorphous organic material.

Recently, Pashley et al (1987) developed a technique, involving ultrasonication and high resolution SEM, to investigate the ultrastructural composition of smear layers produced through mechanical sanding and dental handpiece manipulation. They stated that smear layers consist of heterogeneous populations of minute particles of tooth substance which are burnished together so tightly that they can not be resolved by routine SEM techniques. Although a wide variety of particle sizes were detected, each particle making up the smear layer was found to be composed of a globular structure 0.01 to 0.1 μm diameter. Pashley suggested that these 'elementary globular units' may represent an important basic substructural unit of the dentine matrix.

The exact histological structure of smear layer has not been completely elucidated nor have the clinical implications of its existence been fully investigated. None the less, several modifications of instrumentation techniques have been promoted in order to routinely remove smear layers from restorative cavity preparations and from within root canals.

Gutiérrez & Garcia (1968) noted, in their light microscopy investigation of mechanical preparation of root canals, that root canal walls were "smoother, more polished and the subjacent

dentine exhibited a sugared appearance" when EDTA¹ (Østby's formula) was used. Fromme & Reidel (1972) using SEM, also noted the "clean dentine surface" produced by the use of Largal Ultra² during instrumentation. However, these studies did not specifically identify the smear layer as a discrete entity.

McComb & Smith (1975) first reported the complete removal of smear layer from instrumented root canals following irrigation with REDTA (15% EDTAC). Furthermore, they showed that an unbuffered 15% EDTA solution, without a surfactant (cetramide), was inconsistent in both the degree and frequency with which it removed the smear layer. Hydrogen peroxide, sodium hypochlorite and polyacrylic acid did not remove the smear layer.

Leidel & Eriksen (1979) showed that 15% EDTA totally removed the smear layer from ground dentine surfaces but only partially from enamel. A 50% citric acid based cleansing agent (EpoxyLite 9060) and 37% phosphoric acid removed the smear layer from both enamel and dentine. Water, Tublicid³ and hydrogen peroxide had no significant effect upon the smear layer.

Brännström et al (1979a; 1980) investigated the ability of water, hydrogen peroxide, benzalkonium chloride, EDTA and other formulated products to remove the smear layer from dentine surfaces, in vivo. Their intention was to identify a technique which would selectively remove smear layer from the cut dentine surface without simultaneously removing it from the underlying dentinal tubules. They found that only 0.2% EDTA, in combination with an experimental surface-active solution, satisfied this aim.

Wayman et al (1979) evaluated the use of lactic acid, citric acid and sodium hypochlorite as root canal irrigants. They concluded that a 10% solution of citric acid followed by 2.5% NaOCl, when combined

1 15% ethylenediaminetetra-acetic acid, disodium salt ; 0.67M solution
 2 15% EDTA with 0.84% cetramide (cetyl trimethylammonium bromide) pH 7.3
 3 0.1% chlorhexidine gluconate; 9% dodecylaminoethyl glycine aq. solution

with a final citric acid flush, produced the cleanest canal walls and consistently removed the smear layer. However, they observed the formation of a crystalline deposit on the dentine surface after citric acid irrigation. Similar findings were also reported by Rocca & Duprez (1985) and Blackler (1983).

Goldman et al (1981) examined the cleansing effects of 1% TEGO¹, 5.25% NaOCl and REDTA solutions delivered through a perforated needle for irrigation of the root canal. Their results indicated that only REDTA could completely remove the smear layer.

Goldman et al (1982) studied the efficacy of 5.25% NaOCl and REDTA irrigating solutions singly or in combination. They concluded that canal debridement was more effective if NaOCl was used as the irrigant during instrumentation but that the two solutions used sequentially as a final flush irrigation had a synergistic effect which completely debrided the root canal of both organic and inorganic debris including the smear layer.

Following on from the work of Goldman and co-workers, Yamada et al (1983) further evaluated the significance of irrigating solution sequence on the efficacy of root canal debridement. They confirmed that the optimal combination was 5.25% NaOCl, as the irrigant during instrumentation, followed by a final flush with 10 mls of 15% EDTAC and 10 mls of 5.25% NaOCl. This combination most consistently and completely removed all debris from the root canal, including the smear layer. They suggested that the sequence of use was also significant.

Baumgartner et al (1984) examined the irrigant efficacy of saline, 5.25% NaOCl and 50% citric acid solutions. When a combination of irrigants was evaluated, both solutions were used after each sequentially sized file rather than as a final high volume flush. Their results showed that combinations of NaOCl and citric acid,

¹ 1% dodecyldiaminoethyl glycine - an ampholytic detergent

irrespective of the order in which they were used, were capable of removing both the superficial debris and the smear layer.

Baumgartner & Mader (1985) also recently investigated the efficacy of 5.25% NaOCl and 17% EDTA irrigants during root canal debridement. Unlike Yamada et al (1983), this group alternated each solution throughout the entire instrumentation phase and did not restrict the experimental irrigation to a final flush procedure. In general, their data duplicated and confirmed those of Yamada with the exception that the sequence of irrigant use was not found to be significant.

Several studies, using sonic and ultrasonically energized instrumentation, have also demonstrated smear layer removal from root canals, in vitro, independent of the nature of the irrigant solution (Cunningham & Martin, 1982; Cameron, 1983; Cymerman et al, 1983; Goodman et al, 1985; Rocca & Duprez, 1985; Haikel & Allemann, 1988).

Cameron (1983) also showed that 'static' ultrasonication of the root canal with 3% NaOCl would completely remove the smear layer after 3 minutes activation without the need of an irrigant 'flow'.

However, several recent studies have reported inconsistencies in the degree and reliability of ultrasonication alone and topically applied conditioning agents, which do not contain EDTA, to completely remove the smear layer from instrumented root canal walls, in vitro. (Ram, 1980; Langeland et al, 1985; Rocca & Duprez, 1985; Rome et al, 1985; Baker et al, 1988; Cameron, 1988; Meryon et al, 1988).

Various empiric hypotheses have been proposed in order to justify the adoption of these recommendations which specify either the routine removal of the smear layer or its replacement within endodontically treated teeth.

2.3 Clinical Significance of Smear Layer in Endodontics

The clinical significance of the smear layer in endodontically treated teeth has not yet been well established (Cotton, 1984). Various conflicting opinions within the literature present the clinician with a dilemma as to the pathological significance of this structure and indications for its removal (Baumgartner et al, 1984). These opinions have largely been extrapolated from speculative and personal attitudes rather than from objective, clinically relevant research (Gwinnett, 1984; Mader et al, 1984).

One predominant view claims that the smear layer may be a liability to the extent that it may harbour bacteria and other noxious substances (Brännström, 1984). In direct contradiction to this view, another states that the smear layer may represent a natural cavity liner, which is capable of isolating or sealing the root canal, thereby preventing the expression of detrimental effects on sub-adjacent vital tissues (Lester & Boyde, 1977; Dippel et al, 1981; Pashley, 1985).

Lester & Boyde (1977), using SEM photomicroscopy, showed that the particle size of a ZOE based root canal sealer (Tubliseal) was larger than the maximum dentinal tubular diameter and therefore that the sealer was physically incapable of penetrating into the dentine. In contrast to this finding, several authors have claimed that removal of the smear layer permits better adaptation of endodontic materials and cements to root canal dentine (Goldman et al, 1982; 1984a,b,c; White et al, 1984; 1987; Buchanan et al, 1985).

There are many conflicting opinions regarding the desirability of removing the smear layer, with respect to dentine adhesion in general dentistry (Powis et al, 1982; Bowen, 1984; Lacefield et al, 1985). However, these and similar studies are often selectively cited to either support or refute hypotheses regarding the desirability of smear layer removal from root canals.

Powis et al (1982), and later Lacefield et al (1985), investigated the effects of conditioning agents on the adhesion of glass ionomer

materials to dentine. They concluded that pretreatment of the dentine to remove smear layer with strongly acidic or low molecular weight chelating agents, such as EDTA, did not result in a significant improvement in bond strength. However, Powis demonstrated a significant increase in bond strength when high molecular weight, multifunctional substances such as polyacrylic and tannic acid were used to remove the smear layer from dentine.

Bowen et al (1984) showed that removal of the smear layer alone resulted in the reduced adhesion of restorative materials to dentine. However, they also demonstrated that smear layer removal followed by an experimental coupling agent, which restored an artificial 'smear layer', resulted in significant increases in bond strength.

Goldman and co-workers have advocated removal of the smear layer from the root canal in order to enhance the tensile strength of cemented posts. Using SEM, Goldman et al (1984a) showed that unfilled composite resin tags would penetrate into the dentinal tubules following post cementation if the smear layer was removed. Goldman et al (1984b; c) concluded that the greatest tensile post strength was achieved when the smear layer had first been removed from the root canal. However, these studies also showed that the increase in tensile strength was only statistically significant for long posts cemented with composite resin but not with other traditional cements.

White et al (1984; 1987) speculated that their demonstration of sealer penetration into dentinal tubules following smear layer removal may indicate an improvement in the biological seal of an obturated root canal. However, this has not yet been validated by objective research.

Buchanan et al (1985) reported that vertically condensed warm gutta percha was occasionally seen penetrating into the dentinal tubules of root canals with the smear layer removed when viewed under SEM.

White et al (1985), using fluorescent microscopy, concluded that removal of the smear layer allowed penetration of the tubules by sealers and filling materials up to 1.5 mm from the canal space. However, the validity of this technique has not been established, with respect to a possible unrelated hydraulic movement of the dye during obturation.

Cergneux et al (1986) used a dye diffusion technique to assess the influence of the smear layer on the apical seal of endodontically treated teeth. They were unable to show any statistically significant difference in dye leakage either with or without smear layer.

Pashley et al (1988) and Tao & Pashley, (1988a) showed that removal of the smear layer resulted in a reduction in the shear bond strength of composite to dentine but also showed that smear layer improves the bond strength significantly in the presence of a physiological pulpal pressure (Tao & Pashley, 1988b).

Another important consequence of smear layer removal is that the surface area of exposed dentinal tubules may increase by 10 to 25% (Garberoglio & Brännström, 1976; Pashley et al, 1978b). This alteration in the available "diffusional surface area" is known to greatly increase fluid movement through dentine by filtration (Reeder et al, 1978; Boyer & Svare, 1981; Pashley et al, 1981c; 1983a; 1988) and, to varying degrees, also by diffusion (Merchant et al, 1977; Pashley & Livingston, 1978; Pashley et al, 1978b; Boyer & Svare, 1981; Dippel et al, 1984 and Pashley & Galloway, 1985).

Although many authors agree that the presence of a smear layer may prevent or inhibit bacterial invasion of the dentinal tubules by micro-organisms (Vojinovic et al, 1973; Mjör, 1974; Michelich et al, 1980; Gutiérrez et al, 1982; Diamond & Carrel, 1984; Meryon et al, 1986b) others have suggested that the smear layer is still permeable to the products of bacterial metabolism and decomposition, and therefore may provide a reservoir of potential irritants (Brännström & Nyborg, 1974; Bergenhöltz, 1977; Michelich et al, 1980; Pashley, 1984; Williams & Goldman, 1985).

Numerous studies have shown that many restorative materials leak and allow bacteria to migrate between the cavo-surface margin and the restoration (Skogedal & Eriksen, 1976; Brännström et al, 1979b; Mejare et al, 1979; Qvist, 1980; Bergenhöltz et al, 1982; Brännström, 1982, 1984; Mejare et al, 1987). Brännström & Nyborg (1973; 1974) and Brännström et al (1979b) concluded that bacteria within the smear layer of coronal cavity preparations were both viable and capable of producing inflammatory changes within the underlying pulpal tissue.

Several in vivo studies have histologically demonstrated that carious dentine (Mjör & Tronstad, 1972; Bergenhöltz, 1977; 1981) and plaque (Bergenhöltz & Lindhe, 1975; Bergenhöltz & Warfvinge, 1982), placed into restorative cavities, are capable of evoking inflammatory changes in the sub-adjacent coronal pulp tissue. Similarly, Takahashi et al (1970); Torabinejad & Kiger (1980) and Maita et al (1986) have experimentally demonstrated the ability of necrotic root canal debris to induce inflammation within periapical tissues. However, these studies were conducted without specific reference to the removal or presence of the smear layer.

Recent investigations have established (Andreasen, 1980; 1981a,b; Lindskog et al, 1983; 1986; Hammarström et al, 1986a,b; Tronstad, 1988) that the repair of surface root resorption, following traumatic injury, was adversely affected if the 'pulpal' dentinal tubules were invaded by bacteria. The removal or neutralization of these micro-organisms was essential to preventing the development of external inflammatory resorption (Andreasen, 1981a,b; Hammarström et al, 1986a). The influence and potential protective role of the smear layer in this regard remains speculative. However, Lindskog et al (1986) have recently demonstrated in vivo that significantly more ankylosis, but less inflammatory root resorption, occurred in avulsed teeth which were replanted and subsequently debrided using ultrasonics.

Clinically, it has been shown that traditional therapeutic agents, such as calcium hydroxide and root canal sealers, may be unexpectedly cytotoxic to periodontal membrane cells if placed within the root canal immediately following traumatic injury to the periodontium (Andreasen, 1981b; Andreasen & Kristerson, 1981; Hammarström et al, 1986c). The significance of the smear layer in modifying this toxicity has not been evaluated.

Callis and Santini (1985) histologically evaluated endodontically treated teeth implanted into rats. They observed that the inflammatory response was significantly more intense adjacent to areas of dentinal tubules which were "patent", i.e. the smear layer was removed. They speculated that dentinal tubules not occluded following obturation, either by root canal sealer or smear layer, may remain immunologically inductive to polymorphonuclear leukocytes within the periodontal tissues thereby perpetuating periapical disease.

The above in vitro and in vivo studies indicate that dentinal tubules within the root canal are capable of containing and transporting biologically active factors which may potentially influence the reparative response of adjacent periodontal tissues and that the effects of these factors or inductants are capable of being expressed at some distance from their source.

The clinical merits of smear layer retention or removal from the root canal of endodontically treated teeth have not been conclusively established in other than an extrapolated or hypothetical sense.

2.4 Eugenol and Zinc Oxide Eugenol containing Materials

Eugenol, 4-allyl-2-methoxyphenol (App. 3.1), is an extract of clove oil widely used as a therapeutic agent in dentistry, medicine and homeopathy. Its popularity has been empirically based upon an extensive history of positive clinical experience or efficacy. Eugenol, in combination with zinc oxide, has enjoyed a unique and popular position amongst dental clinicians as the basic component of many anodyne, antiseptic and restorative material formulations. Specific to this study, eugenol has been extensively used as the base for many current endodontic sealer and sedative formulations.

2.4.1 Eugenol pharmacokinetics

Eugenol reacts with zinc oxide to form an amorphous crystalline molecule, zinc eugenolate. There is a large stoichiometric excess of zinc oxide in all clinically useful ZOE mixtures, with liquid: powder ratios of the order 1: 5 (Wilson & Batchelor, 1970; Douglas, 1972; Wilson et al, 1973). The set material is thought to be composed of a conglomerate of unreacted zinc oxide particles embedded within a zinc eugenolate matrix (Wilson & Batchelor, 1970; Wilson et al, 1973). Reportedly, there is no 'free' eugenol available for simple diffusion out of the mass once the setting reaction is complete (Douglas, 1972; Wilson et al, 1973; Crisp et al, 1976; Becker et al, 1983; Hume, 1984a).

Wilson & Batchelor (1970) found that eugenol was released from ZOE mixtures through a process of hydrolysis. They proposed that an excess of water shifted the reaction equilibrium such that the eugenolate matrix was disrupted, which in turn resulted in the subsequent liberation of 'free eugenol' molecules and the formation of an insoluble zinc hydroxide precipitate. They concluded that when freshly mixed ZOE mixtures were placed in direct contact with an aqueous phase, free eugenol would be available only during the setting reaction (before chelation) and after setting, via hydrolysis of the eugenolate matrix.

Several investigations have demonstrated that eugenol is rapidly released from ZOE mixtures following immersion in an aqueous solution, and that the release rate declines exponentially with time (Wilson & Batchelor, 1970; Wilson et al, 1973; Becker et al, 1982, 1983; Hume, 1984a). However, Wilson & Batchelor (1970) and Wilson et al (1973) were not concerned with the initial eugenol release phenomenon but rather with the process of disintegration *per se*.

Becker et al (1982, 1983) determined that the eugenol release rate from ZOE mixtures was greatest during the first ten minutes after immersion. The release rate declined to about one percent of the initial rate by 24 hours, and then continued to decline exponentially thereafter. They also established that the rate of release was highest, during the first day, with the lowest powder : liquid ratio (1 : 2). However, this ratio was too thin to be used clinically. After 24 hours, no significant differences in release rate(s) were attributable to alterations in powder : liquid ratios. If the samples were allowed to set prior to immersion then the release rates were maintained at values much less than those immersed immediately after mixing.

Veaco et al (1982) and Hume (1984a) confirmed that eugenol was also released through dentine from zinc oxide eugenol materials (ZOE), when placed into cavities in human molar teeth, following its progressive hydrolysis at the ZOE/dentine interface only. Hume also noted the formation of an insoluble zinc hydroxide/zinc oxide layer, at the hydrolysis/dentine interface, which appeared to significantly inhibit eugenol availability.

Hume (1984a) concluded that water availability for hydrolysis was the prime determinant of eugenol release from ZOE mixtures. Eugenol is only slightly soluble in water ($K_p = 10^{-3}M$) but readily soluble in lipid solvents (British Pharmacopea, 1980; Martindale, 1982) but considerably more soluble than either the eugenolate or zinc hydroxide. He proposed that the process of 'eugenolate' hydrolysis was the rate limiting step in the bioavailability of eugenol and concluded that the data provided the basis for an explanation of

the paradox between therapeutic and toxic actions of ZOE materials during clinical usage.

Veaco et al (1982) investigated the concentration-time release characteristics of eugenol and ZOE mixtures through coronal dentine when placed into cavity preparations of freshly extracted human teeth, in vitro. The peak eugenol concentration occurred about one hour after placement and thence declined exponentially. They also demonstrated that a pulpal concentration in excess of 10^{-5} M eugenol was maintained from 10 minutes to 8 hours after placement.

Hume (1984a) studied the release dynamics of eugenol from ZOE mixtures following both immersion into saline and placement into coronal cavity preparations of freshly extracted human teeth, in vitro. He found that the peak eugenol release at the pulpal dentine surface was about three orders of magnitude (10^{-3} x) less than that at the salivary or occlusal surface. The peak eugenol concentration within pulpal dentine was 10^{-4} M and remained relatively constant for more than one week, unlike release into aqueous solution.

Hashimoto et al (1988) examined the in vivo release of eugenol from ZOE placed into dentine cavities in direct contact with rat dental pulp tissue. They detected eugenol in pulp tissue within 15 minutes of ZOE placement, reaching maximal concentration within pulp tissue at 30 minutes and progressively declining to base levels by four hours post-operatively. The maximum levels of eugenol in pulp tissue at 30 minutes corresponded to 35 pmol per mg of pulp.

Hume (1984a) demonstrated a tendency for peak release values to bear an inverse relationship to the thickness of the intervening layer of dentine and to the time of peak development. He reported that the pulpal outflow remained within one order of magnitude of its peak level for at least one week after material placement.

Later work by Meryon & Jakeman (1986) confirmed that eugenol release varied inversely with the intervening dentine thickness. Meryon (1988a,b) also found that removal of the smear layer, from

the intervening dentine with 50% citric acid, resulted in a significant increase in subsequent eugenol availability. However, their data was presented in terms of hypothetical "machine units" and could not be correlated with actual eugenol concentrations.

Hume (1984a) suggested that the reduction in eugenol release across dentine could be explained in terms of the "functional wetness" of dentine and, consequently, its reduced potential for hydrolytic interaction with the ZOE material. This hypothesis was based upon the "diffusional surface area" concept proposed by Pashley et al (1978b) [viz : p.13]. The 'diffusional surface area' of human coronal dentine has been independently calculated at about 1% of its overall available surface area (Pashley et al, 1978b; Boyer & Svare, 1981; van der Graaf & Bosch, 1988).

Furthermore, Hume (1984a, 1986) proposed that, since the principal in vitro determinant of eugenol release from ZOE materials was dependent upon the 'functional surface area' of moist dentine available for hydrolysis, it was the intervening layer of dentine which ultimately determined whether a ZOE material would be therapeutic or toxic in its effect upon pulpal tissue in vivo.

2.4.2 Eugenol pharmacology

The therapeutic properties of eugenol containing materials do have recognized scientific and pharmacological bases. These have been primarily attributed to the anti-microbial activity of these materials (Mjör et al, 1977; Bergenhöltz et al, 1982) and their reported potent non-steroidal anti-inflammatory activity i.e., NSAIDs (Dewhirst & Goodson, 1974; Dewhirst, 1980; Hirafuji, 1984; Hashimoto et al, 1988).

Dewhirst investigated the structure activity relationships for the inhibition of prostaglandin cyclo-oxygenase, by various groups of phenolic compounds. Cyclo-oxygenase is an enzyme present in all mammalian cell membranes. It is responsible for conversion of arachidonic acid into several important bio-active compounds (prostaglandins). Prostaglandins are considered to be important non-specific effector mediators in inflammatory reactions (Raisz et al, 1974; Giroud & Willoughby, 1970). They have been strongly implicated as the substances responsible for inducing pain (Torabinejad & Bakland, 1980a,b; Cohen et al, 1985; Perrini & Fonzi, 1985), and bone resorption in both acute and chronic apical periodontitis lesions (Yoneda & Mundy, 1979; Dominguez & Mundy, 1980; Sidhagen et al, 1982; Seymour et al, 1985; Torabinejad & Luben, 1985). Dewhirst (1980) demonstrated that eugenol was a potent inhibitor of cyclo-oxygenase activity and therefore of prostaglandin synthesis. Its activity was approximately equivalent to that of the well established NSAID, indomethacin, at concentrations of 10^{-6} M. Hirafuji (1984) has similarly demonstrated the ability of eugenol and other phenolic compounds to inhibit the cyclo-oxygenase catalysed biosynthesis of various prostaglandin substances in vitro.

Recently, Hashimoto et al (1988) used radioimmunoassay, liquid scintillation counting and thin layer chromatography techniques to measure the in vivo levels of prostaglandin production and tritiated (^3H)-eugenol release into the dental pulps of rats. They found that cyclo-oxygenase products disappeared immediately following the placement of ZOE material into dentine cavities but were not altered

when the cavity was filled with a control zinc oxide-water material. However, one limitation of this study relates to the use of cavities with a direct pulp exposure of the cavity floor.

Eugenol is known to be anti-bacterial in culture (Antrim, 1976; Grossman, 1980; Wennberg, 1980; Ørstavik, 1981; Stevens & Grossman, 1981) and has been reported to be responsible for the persistent in vivo antimicrobial activity of ZOE based root canal sealers (Delivanis et al, 1983; Peters, 1986). However, few studies have examined the concentration(s) necessary to ensure this effect. Newman et al, (1979) tested micro-organisms from dental plaque, including obligate and facultative anaerobic bacteria, for their susceptibility to several clinical antiseptic agents, including eugenol. They found that only a few were sensitive to eugenol concentrations less than 0.7×10^{-3} M, whilst concentrations greater than 2.2×10^{-2} M were required to prevent the growth of all micro-organisms tested.

Whilst eugenol containing materials have enjoyed widespread clinical acceptance, numerous reports and experimental studies have generated concern for a potential imbalance between the perceived advantages versus the potential detrimental effects of these compounds. This concern is based upon several biocompatibility studies indicating that eugenol containing materials are potentially toxic to eukaryotic tissues and various cultured mammalian cell lines (Rappaport et al, 1964; Kawahara et al, 1968; Spangberg et al, 1973; Mjör et al, 1977; Hensten-Pettersen & Helgeland, 1977; Browne & Tyas, 1979; Barnett et al, 1984).

Most reports of detrimental and unpredictable effects relate specifically to the use of eugenol and ZOE mixtures in direct contact with vital tissues (Erausquin et al, 1966; 1967a,b; 1970; Paterson, 1976; Brodin & Ørstavik, 1983; Cox et al, 1987; Watts & Paterson, 1987). However, data have become available which may provide an explanation for the apparent paradoxical therapeutic effects of ZOE materials when applied to intact dentine and the toxic effects of the mixture when applied directly to vital cells or tissues.

Recently, it has been suggested that the blandness of ZOE mixtures, in clinical usage, may be directly attributed to the presence and modifying influence(s) of an intervening layer of dentine between the ZOE material and sub-adjacent vital tissue (Hume, 1984a; Browne, 1985; Hume, 1986; Meryon & Jakeman, 1986a; Watts & Paterson, 1987; Meryon, 1988a,b).

Early work by Rotberg & de Shazer (1966) demonstrated that slabs of dentine were capable of absorbing "significant amounts" of a eugenol liquid applied to them. However, the method of quantification used in this study did not permit accurate calculation of these amounts.

Meryon & Jakeman (1986a) and Meryon (1988a,b) used powdered dentine and 100 to 1000 μm slices of dentine to assess the influence of dentine upon both eugenol release and cellular toxicity, in vitro. They showed that the intervening dentine considerably reduced both the cumulative 24 hour release of eugenol and concomitantly reduced the in vitro cytotoxicity of the four ZOE materials studied.

Direct measurement of eugenol release into and through human coronal dentine (Veaco et al, 1982; Hume, 1984a) showed that peak eugenol release through dentine was approximately a thousand fold less than that into water from the same ZOE mixture. They found that, within 12 hours of placement, a concentration gradient was established across dentine such that the peak eugenol concentration in dentine immediately beneath the ZOE mixture was in excess of 10^{-2} M but, that at the dentino-pulpal junction, this had reduced to a concentration of 10^{-4} to 10^{-5} M or less.

Recently, Meryon (1988a, b) reported that removal of the smear layer, from 100 and 500 μm thick slices of dentine, significantly increased the in vitro toxicity of test ZOE materials. However, in contradiction to Hume (1984a), she concluded that the relatively small increase in eugenol availability following smear layer removal suggested that the relative increase in water availability alone could not completely account for the increase in cytotoxicity demonstrated.

Protein has also been implicated in the protective role of dentine, in vivo. Imai et al (1982) demonstrated a decrease in cellular toxicity of ZOE mixtures with increasing serum concentrations within the nutrient medium. Similarly, Fujisawa & Masuhara (1981) have reported a strong binding avidity between eugenol and serum albumins. They suggested that this interaction within dentine tubules might provide a protective barrier between released eugenol and the pulp.

The toxic effects of ZOE materials have been attributed to zinc ions, eugenol and the combination of both. Zinc is reported to be eluted from specimens only very slowly and in trace amounts (Wilson & Batchelor, 1970; Douglas, 1972). Unfortunately, there was no quantification of the amounts of zinc released and the reaction dynamics of these studies can not be applied to the clinical situation but represent only the disintegration of ZOE immersed in bulk solutions of water.

Kawahara et al (1968) used L-cell monolayer cultures and reported pronounced in vitro cytotoxic effects of metallic zinc and zinc ions. Kawahara et al (1979) later speculated that zinc ions could reach toxic levels in vivo during the decomposition of ZOE materials. However, they concluded that the effects of eugenol would overshadow the toxicity due to zinc ions. Unfortunately, the relevance of this data is questionable as no details were given of either the zinc concentration(s) or exposure times used.

Several investigations have also shown that zinc may be toxic to various cultured mammalian cells at concentrations ranging from 5 to 30 p.p.m. (Leirskar, 1974; Leirskar & Helgeland, 1981; Lucas et al, 1984; Meryon & Jakeman, 1985). Meryon & Jakeman (1986b) quantified the in vitro release of zinc from five restorative materials, during immersion testing, and found that zinc was released from ZOE at greater than 25 p.p.m. but only after 14 days. They attempted to correlate this in vitro data with cell culture work (1985), stating that it was possible for zinc to reach levels (3 to 10 p.p.m.) within the

media which could partly account for the delayed and prolonged in vivo cytotoxicity of ZOE materials. However, in contradiction to this statement, these authors (1985) have concluded that zinc, released from ZOE materials in vivo, was unlikely to lead to toxic effects since "zinc levels of this order are known to be readily adsorbed by the intervening dentine." (Meryon, 1984a; Meryon & Jakeman, 1986b).

Further indirect evidence was presented by Hashimoto et al (1988) in their in vivo study of the influence of ZOE materials on rat dental pulp. They demonstrated that eugenol containing zinc oxide materials did significantly reduce the biosynthesis of cyclo-oxygenase products in irritated dental pulps whilst a control mixture of zinc oxide and water had no effect.

Most investigators have generally attributed the toxicity of ZOE materials to eugenol (Browne, 1985). It has been suggested that it exerts its toxicity either through direct and non-specific hydrophobic interaction with cell and organelle membranes, resulting in cell lysis (Spott & Rosett, 1973; Lindqvist & Otteskog, 1981), or that it may interfere with cellular metabolism at an enzyme level (Cotmore et al, 1979; Lindqvist et al, 1980; Hume et al, 1982; Hume, 1983; 1984a; Suzuki et al, 1985a,b; Fotos et al, 1987).

The toxic effects expressed by cellular contact with eugenol are known to be concentration dependent (Kozam, 1977; Lindqvist & Otteskog, 1981; Hume et al, 1982; Hume, 1983; 1984b; Brodin & Ørstavik, 1983; Seow & Thong, 1986; Fotos et al, 1987). Furthermore, the eugenol concentration developed within a tissue has been shown to be proportional to the rate of its hydrolysis, decreasing with time (Wilson & Batchelor, 1970; Wilson et al, 1973; Ranly et al, 1975; Becker et al, 1982; Hume, 1984a), and dependent upon intervening dentine thickness (Rotberg & de Shazer, 1966; Tyas & Browne, 1977; Hume, 1984a; Meryon, 1984; 1988a,b; Meryon & Jakeman, 1986).

High concentrations, in the order of 10^{-2} M, have been reported to cause protein denaturation, cellular necrosis and the dissolution of striated muscle, on direct contact (Kozam & Mantell, 1978). Sticht &

Smith, (1971) administered intravascular infusions of eugenol to dogs and demonstrated that 10^{-2} M concentrations not only caused a widespread fall in blood pressure, peripheral vasodilation and central nervous system depression, but they also irreversibly damaged capillary membranes and caused coagulation necrosis of the adjacent extravascular tissues.

Ozeki (1975) found that 1.2×10^{-3} M anaesthetized crayfish whilst Kozam (1977) showed that levels greater than 5×10^{-4} M would irreversibly block impulse transmission along frog sciatic nerve preparations. Brodin & Ørstavik (1983) confirmed these non-mammalian results with work on rat phrenic nerves. They found that concentrations in the order of 10^{-4} M would block impulse transmission and that nerves dissected free of fatty tissue would be irreversibly blocked. Similar results were also reported by Trowbridge et al (1982). It is not possible to conclude from *in vitro* studies whether these pharmacological effects would be therapeutically or toxically expressed during normal clinical usage.

The toxic effects of eugenol on cultured mammalian cells have also been extensively examined. Cotmore et al (1979) have shown that eugenol maximally inhibits rat liver mitochondrial respiration at 1.67×10^{-4} M and, Spott & Rosett (1973), that it labilizes lysosomal membranes in homogenates of dental pulp. Lindqvist & Otteskog (1981) found that human fibroblast growth was inhibited at an IC_{50} of 5×10^{-4} M, and survival prevented at concentration of 4×10^{-3} M or greater.

Hume et al (1982) reported slightly more sensitive results for mouse liver, rat and rabbit dental pulp homogenates with IC_{50} and LD_{100} values of 10^{-6} M and 6×10^{-4} M, respectively. Hume (1984b) later showed that human pulp and mouse fibroblast monolayers were similarly affected but at slightly more concentrated values, namely an IC_{50} of 10^{-4} M and a LD_{100} of 10^{-3} M, indicating that there was some species and tissue specificity. He also established that eugenol toxicity was time- as well as concentration-dependent. He proposed

that suppression of cellular respiration by eugenol kills cells if it continues for about 24 hours or more.

Seow & Thong (1986) also reported time-dependent inhibition of human PMN function and viability. They found that eugenol concentrations greater than 10^{-3} M were lethal and 10^{-4} M was inhibitory to PMN function, as measured by adherence, after incubation for as little as five minutes. At concentrations less than 10^{-5} M, PMN function was apparently stimulated provided contact was limited to 5 minutes. However, incubation for longer intervals with 10^{-5} M eugenol resulted in the progressive diminution of PMN function. Unfortunately, neither the methodology nor statistical analyses were sufficiently detailed to allow relevant clinical conclusions to be drawn.

In a recent study, Fotos et al (1987), examined the effects of eugenol on human PMN function, as measured by cellular migration and chemiluminescence assay. They similarly reported that eugenol concentrations of 6.6×10^{-2} M and 6.6×10^{-3} M were lethal to PMN. However, whole cell and free myeloperoxidase chemiluminescence assays indicated that enzyme inhibition was complete at 6.6×10^{-5} M, and that significant enzyme inhibition occurred down to 6.6×10^{-6} M. In cell-free enzyme studies, full chemiluminescence was regained at 6.6×10^{-7} M or lower, but this recovery was not apparent in whole cell studies. This observation implies that other factors in addition to enzyme inhibition may be affected by eugenol in vitro. They concluded that PMN membrane function is compromised at eugenol concentrations much lower than those likely to occur in periodontal and endodontic applications (Hume, 1984a).

In addition to direct cytotoxicity investigations, there are also reports of both allergenic (Barkin et al, 1984; Hensten-Pettersen et al, 1985; Gazelius et al, 1986) and potentially mutagenic responses to eugenol (Woolverton et al, 1986). Block and his associates (1978a,b) have shown that pulp tissue treated with a variety of endodontic sealer pastes, including eugenol based sealers, are capable of inducing both

humoral and cell mediated immune responses in dogs. However, studies using sealers alone as immunogens have not been able to reproduce these results in guinea pigs (Campell et al, 1978), dogs (Torabinejad et al, 1979), or rabbits (Stuart et al, 1979; Grossman & Lally, 1982). Hence, eugenol has been shown to be haptenic, stimulating or enhancing immunological responsiveness, but there is limited evidence to suggest that immunological factors, *per se*, are solely responsible for these effects (Roitt & Lehner, 1983; Chapel & Haeney, 1984; Hensten-Pettersen et al, 1985).

The above studies indicate that eugenol has the potential to be both therapeutic and cytotoxic to mammalian cells. Its properties are expressed in a time- and concentration-dependent manner. Toxic effects are reported to occur across a broad range of concentrations (10^{-6} M to 10^{-2} M) which are dependent upon individual species and tissue sensitivities. Prokaryote organisms appear to be less sensitive to eugenol than mammalian eukaryotic cells.

Based upon these bioavailability data, derived from coronal dentine studies, it would appear reasonable to propose that eugenol concentrations may be greater within root dentine if the smear layer were to be removed. Consideration should therefore be given to the potential increase in the pharmacologic and cytotoxic effects of eugenol based materials which could result from the routine removal of smear layer from endodontically treated teeth.

CHAPTER 3

AIMS AND SCOPE OF STUDY

The aims of this research were :

1. to determine the rate of eugenol outflow (diffusion) through root dentine of endodontically treated teeth, obturated with a ZOE based root canal sealer and laterally condensed gutta percha.
2. to determine the mean eugenol concentration gradient across root dentine, immediately following the moment of peak outflow.
3. to examine the possible influences on rate release kinetics of age, sex, post-extraction interval, irrigant volume, and lateral internal surface area available for diffusion from each specimen.

CHAPTER 4

MATERIALS AND METHODS

Extracted human teeth were prepared and examined by one of two techniques, subsequent to endodontic instrumentation.

Specimens were either obturated with a radioisotopically labelled sealer, then analysed by beta (β^-) liquid scintillation spectrometry (LSC), or the canals were left patent following instrumentation and qualitatively examined under SEM.

4.1. SPECIMEN COLLECTION

Specimens were selected according to strict protocol requirements, in an effort to minimize natural variation in the quantity and quality of dentine. Thirty-eight freshly extracted, single rooted, human teeth were collected satisfying the following criteria :

- Mandibular premolar teeth indicated for orthodontic extraction
- Homologous, bilateral pairs
- Caries and restoration free
- Periodontium within normal limits
- No obvious pulpal calcification
- Single canal, straight and with complete apical development
- Essentially conical root and round in cross-section
- Between the ages 12-25 years
- Completed case history documentation

Clinicians were requested to adopt certain precautions during extraction in order to minimize root surface damage :

- Forceps only extraction
- Single application of forceps if possible
- Primarily rotational movements
- Minimal force necessary

If unable to follow these instructions the extraction was recorded as complicated and the specimen and its pair discarded from the study.

Clinicians were also required to complete the survey form, as below, detailing a history of each tooth so that possible influences due to these factors could later be analysed against variation(s) about the mean(s) of Group I and Group II.

SMEAR LAYER - ITS INFLUENCE ON ROOT DENTINE PERMEABILITY
M.D.Sc. RESEARCH PROJECT - D G H CABLE

Case No:

Date :

DRN :

.....

DATE OF BIRTH

(/ /)

SEX

M / F

City of residence between 7-15 years

.....

TOOTH (FDI)

[:]

TIME AT WHICH EXTRACTED

am/pm

EXTRACTION COMPLICATED

Yes / No

Fig 4.1: Case History Form

Immediately after extraction, each tooth was placed into Dulbecco's[§] phosphate buffered saline¹ (pbs) and stored in a fridge at 4 °C, until its homologous contralateral pair was available for study.

Suitable teeth were randomly allocated to one of the following two groups:

Group I	Control Group - Smear layer present Irrigation with Miltons solution only (N=19)
Group II	Experimental Group - Smear layer removed Irrigation with 15% EDTAC solution (N=19)

These groups were further sub-divided according to the method of analysis :

Group A	Liquid Scintillation Spectrometry	(N=34)
	Diffusion release study :	n = 20 teeth (paired)
		n = 4 control teeth
	Peak dentine concentration study :	n = 10 teeth (paired)
Group B	Scanning Electron Microscopic study	(N=4)
	Group I teeth :	n = 2
	Group II teeth :	n = 2

§ ... Dulbecco's Phosphate Buffered Saline without calcium or magnesium¹

200.0	mg/l	KCl
200.0	mg/l	KH ₂ PO ₄
8.0	gm/l	NaCl
1.15	gm/l	Na ₂ HPO ₄ (anhydrous)

4.2 SPECIMEN PREPARATION

Teeth were contact radiographed on Kodak DF 58 (D speed) film¹ using a Siemens² Heliodent-70, at 0.12 seconds. Both mesial and buccal views were taken in order to confirm that the canal(s) were patent and apices closed; that they exhibited no significant curvature; and that there were no obvious signs of pulpal calcification or secondary dentine formation.

Each specimen from Group A was positioned in an 8 mm hole, drilled through the hard screw cap of a polyethylene scintillation vial³, and secured to the cap with Inlay wax⁴ (Class 1, type II). Once fixed into position, each specimen was permanently cemented into place with a rapid setting epoxy resin⁵ (Fig. 4.2). The assembly was then left to stand at room temperature until the resin had completely set.

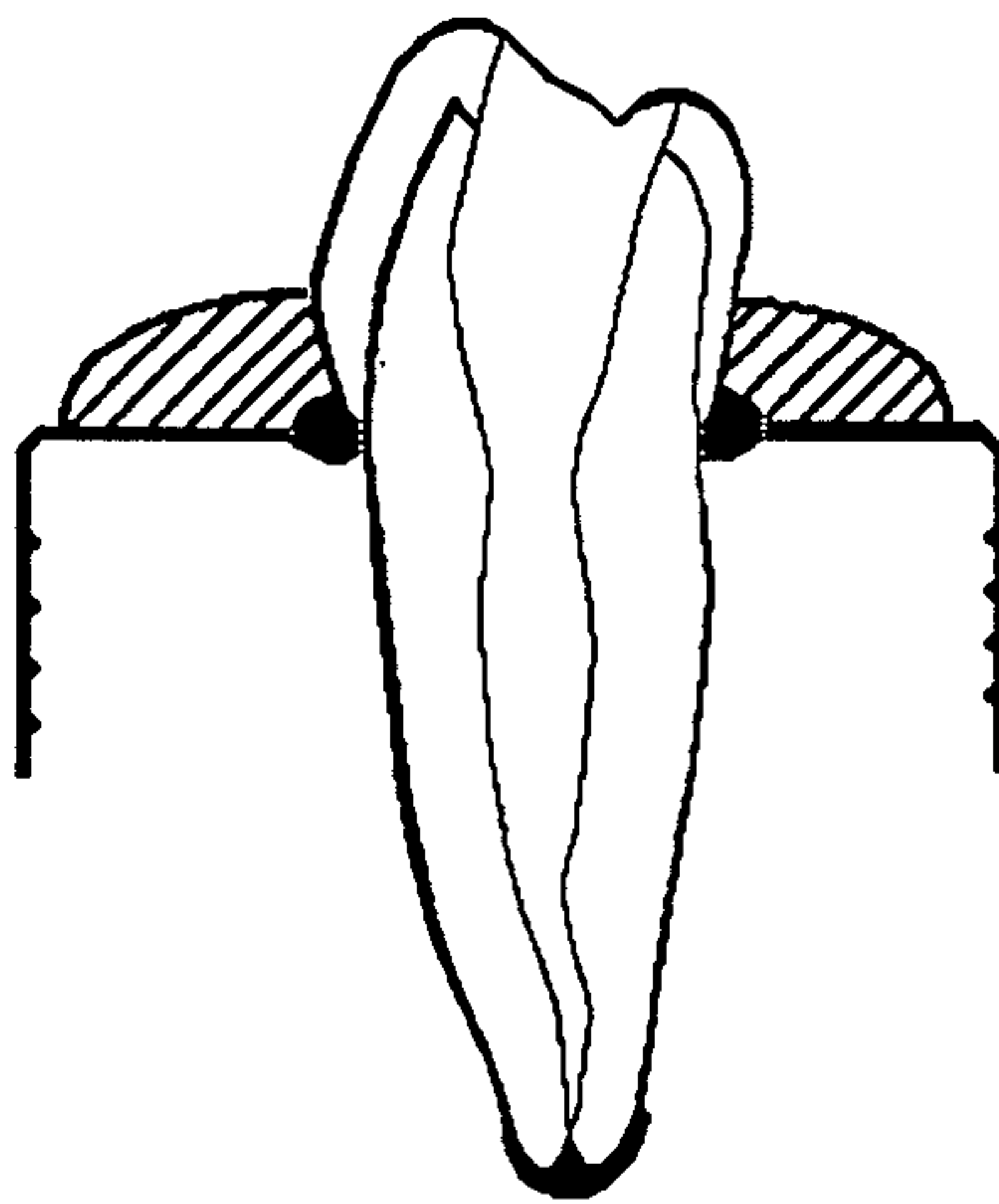


Fig 4.2: Vial-cap Assembly

-
- 1 Eastman Kodak Co. - Rochester, N.Y., U.S.A.
 - 2 Siemens (70kV/7mA) - West Germany
 - 3 Beckman - Mt Waverly, Vic., Australia.
 - 4 Kerr - Emeryville, CA., U.S.A.
 - 5 Selleys Chemical Co. - Padstow, N.S.W., Australia

4.2.1 ENDODONTIC PREPARATION

Routine endodontic access was established through the crown of each specimen pair using a high speed Cardia bur¹ (#020.012) and copious water spray. Care was taken to prepare both access cavities for each pair as equivalently as possible. The cusp tips were lightly reduced to ensure a stable working length reference point. The pulps were then grossly extirpated with barbed broaches² (xf, f, m), and each tooth returned to pbs solution until both specimens from each pair were ready for mechanical preparation.

The working length was determined as the distance from the buccal cusp to a point 1.0 mm short of that which the tip of a #10^k file was just visible at the apical foramen.

The apical 2-3 mm of each root was dried with gauze and painted with a layer of domestic nail enamel (with hardener)³. This step was repeated following mechanical preparation and, for a third time, immediately after obturation but prior to vial inversion at the commencement of permeability analysis for each specimen pair.

The method of biomechanical preparation used was based on the technique currently taught undergraduate students of the University of Sydney. This involved a 'serial' or 'step-back' method without the use of rotary instrumentation (Schilder, 1974).

An apical seat was established at the working length to a minimum size #30^k Master Apical File (MAF), and the canal then serially flared in one (1) mm step-back intervals to a standard final flare size of a #70^k file. The range of M.A.F. sizes was #30^k to #45^k, the mode was #45^k (Table A1.2).

A consistent irrigation technique was used during preparation, both in respect of volume and formulation. The irrigant during

1 Komet (#806.314.198) Brasseler - Lemgo, West Germany.
 2 CC-Cord - München, West Germany
 3 Artmatic - Brooklyn, NY, U.S.A.

preparation was a 1.0% Sodium Hypochlorite (Miltions[§] solution)¹. The following details of instrumentation and irrigation were recorded on the form below for later statistical analysis (Fig. 4.3).

CASE No :

WORKING LENGTH :

IRRIGATION VOLUME :

FINAL FLUSH : Miltions EDTAC

M.A.F.

DATE : OBTURATION TIME :

Fig. 4.3: Instrumentation Record

Each specimen, within a pair, was instrumented and irrigated in a blind and equivalent manner until preparation was completed according to conventional clinical criteria. At this point, each specimen was randomly allocated into one of the two experimental groupings, as previously described (vid: 4.1).

It was intended that the principal variable in this study would be either the presence or absence of smear layer. Smear layer removal was accomplished using the final flush irrigation regime advocated by Yamada et al (1983).

§ ...

Miltions Solution¹

1.0% available Chlorine from NaOCl

16.5% NaCl

1

Miltions Pharmaceutical Company - Sydney, Australia

Group I teeth (Smear present) were irrigated, as a final flush, with 20 mls of Miltons only, whilst Group II teeth (Smear removed) were irrigated with 10 mls of 15% EDTAC [§] followed by 10 mls of Miltons.

Specimens were then further sub-divided into two (2) sub-groups (Gp A & B) based upon the intended method of analyses (vid: p. 45).

Group A specimens (N=34) were obturated with gutta percha and Proco-Sol sealer labelled with tritium (³H). The rate(s) of eugenol diffusion or outflow (N=24, including 4 negative control specimens) and peak dentine concentration of eugenol (N=10) were determined by LSC (vid : 4.3.1).

Group B specimens (N=4) were qualitatively examined under SEM to confirm the experimental irrigation protocol's successful removal of smear layer from the root canal of Group II specimens (vid: 4.3.2).

Four (4) control teeth were instrumented and irrigated in a manner identical to the Group A specimens ($\pm$ smear layer).

Two (2) of these were obturated with the tritium-labelled sealer and their entire exposed root surfaces then coated with nail varnish, as negative (apical) leakage controls. The other two (2) control teeth were obturated with conventional unlabelled sealer and their roots left uncoated. The latter teeth served as processing or technique controls in order to assess if any consistent radio-active cross-contamination occurred during specimen manipulation.

§ ...

15% EDTAC solution according to Ostby's formula (1957)¹

100 mls	Distilled water
17.0 gm	Di-sodium salt of Ethylene-diamino-tetra-acetic acid ² (analytical)
0.84 gm	Cetramide ³ (B.P.)
titrate	5N Sodium Hydroxide ⁴ till pH = 7.5 (approx. 9.25 mls)

-
- 1 Grossman, L.I. (1981)- Endodontic Practice; 10th Ed.; Lea & Febiger : p230.
 - 2 Ajax Chemicals (Univar) - Sydney, Australia
 - 3 Ajax Chemicals (Unilab) - Sydney, Australia
 - 4 Ajax Chemicals (Univar) - Sydney, Australia

4.2.2 OBTURATION

Immediately following completion of canal preparation, each pair of Group A specimens (N=24) were prepared for obturation with gutta percha and a zinc oxide eugenol based root canal sealer (Proco-Sol)¹ using a lateral condensation technique.

During pilot work, repeated trial mixes of Proco-Sol sealer were made with various powder and liquid ratios, measured by weight, in order to establish an accurate and standardized ratio satisfying Grossman's criteria. The following powder : liquid ratio ($\approx 5 : 1$) was found to best satisfy these criteria ; i.e.

0.18 $\pm$ 0.005 g Procosol powder : 38.5 $\pm$ 0.05 mg eugenol (w/w), or
 0.18 $\pm$ 0.005 g Procosol powder : 35.0 μ L $\pm$ 0.3% eugenol (w/v).

All eugenol used for obturation was tritium (^3H) labelled by catalytic proton exchange². The tritium activity concentration (A_0) of the stock eugenol solution was determined by LSC, during October 1987, as $A_0 = 164.5 \times 10^7 \text{ Bq.mole}^{-1}$ or 44.5 mCi.mole⁻¹ (App. 2.2).

This was the equivalent of $39.5 \times 10^4 \text{ Bq}$ or 10.68 μCi per 0.218 gram mix of sealer. Approximately 10 to 20 mg of sealer were placed into each tooth at obturation. This amount of sealer approximated 10 to 20 micromoles of eugenol or an $A_c = 10^2 \text{ Bq}$ ($10^{-2} \mu\text{Ci}$) per canal.

A standardized gutta percha master cone³ was selected which fitted to length with slight resistance to removal, and which permitted spreader penetration to within 2-3 mm of the apical stop. The canals were dried with paper points⁴ and the apical 2 to 3 mm of the root painted with a second coat of nail enamel.

1 Syntex Dental Products - Valley Forge, U.S.A.
 2 Du Pont N.E.N. Products (NET-262) - Boston, MA, U.S.A.
 3 Progress Points (Rudolf Gunz) - Sydney, Australia.
 4 Endo-Aid (Sure Products) - Gurodanji, Korea.

The apical third of each master cone was lightly coated with sealer prior to placement. Sealer was also applied to the canal walls using a dry paper point prior to insertion of the master cone. A #20 finger spreader¹ was used to laterally condense fine-fine and medium-fine gutta percha accessory points². The entire obturation procedure was conducted with each specimen mounted in a blank vial, and an effort made to obturate each specimen of the pair equivalently.

Immediately after completion of obturation, excess gutta percha was removed to the C.E.J. with burn-out instruments and the access sealed with a cotton-pellet and Cavit-W. Once the Cavit had set firmly, sticky wax³ was liberally applied over the crown, epoxy resin and vial cap to ensure a complete hydraulic seal. The assembly was then removed from the blank vial and the root closely inspected for any signs of sealer extrusion. The apical 2 to 3 mm of each root was then recoated with a third layer of nail enamel and allowed to air dry prior to proceeding with the final preparation for analysis.

1 CC-Cord - München, West Germany.
2 Progress Points (Rudolf Gunz) - Sydney, Australia.
3 Kerr - Emeryville, CA., U.S.A.

4.3 METHODS OF ANALYSES

Each tooth was instrumented in one of two ways (Groups I and II), as previously described (vid: 4.2), and then analysed by either LSC subsequent to obturation (Group A), or by SEM following instrumentation but without obturation (Group B).

4.3.1 LIQUID SCINTILLATION SPECTROMETRY

Liquid scintillation spectrometry (LSC) was used to trace the amounts and rates of tritium released through root dentine into pbs solution washing the periodontal root surface. LSC was also used to determine the peak concentration of labelled eugenol across mid-root dentine and to calculate the Specific Activity concentrations of stock tritiated (^3H)-eugenol ($^3\text{H-E}$) and tritiated (^3H)-root canal sealer.

4.3.1a TRITIUM DETERMINATION FROM pbs ELUTION SAMPLES

The obturated 'tooth/vial cap' assembly was removed from the blank vial used during obturation (Fig. 4.4) and returned to its original numbered vial, to which 10 mls $\pm 0.1\%$ of fresh pbs solution had been added. The tooth/vial assembly was then inverted so that the root of each tooth was fully immersed in pbs solution (Fig. 4.5).

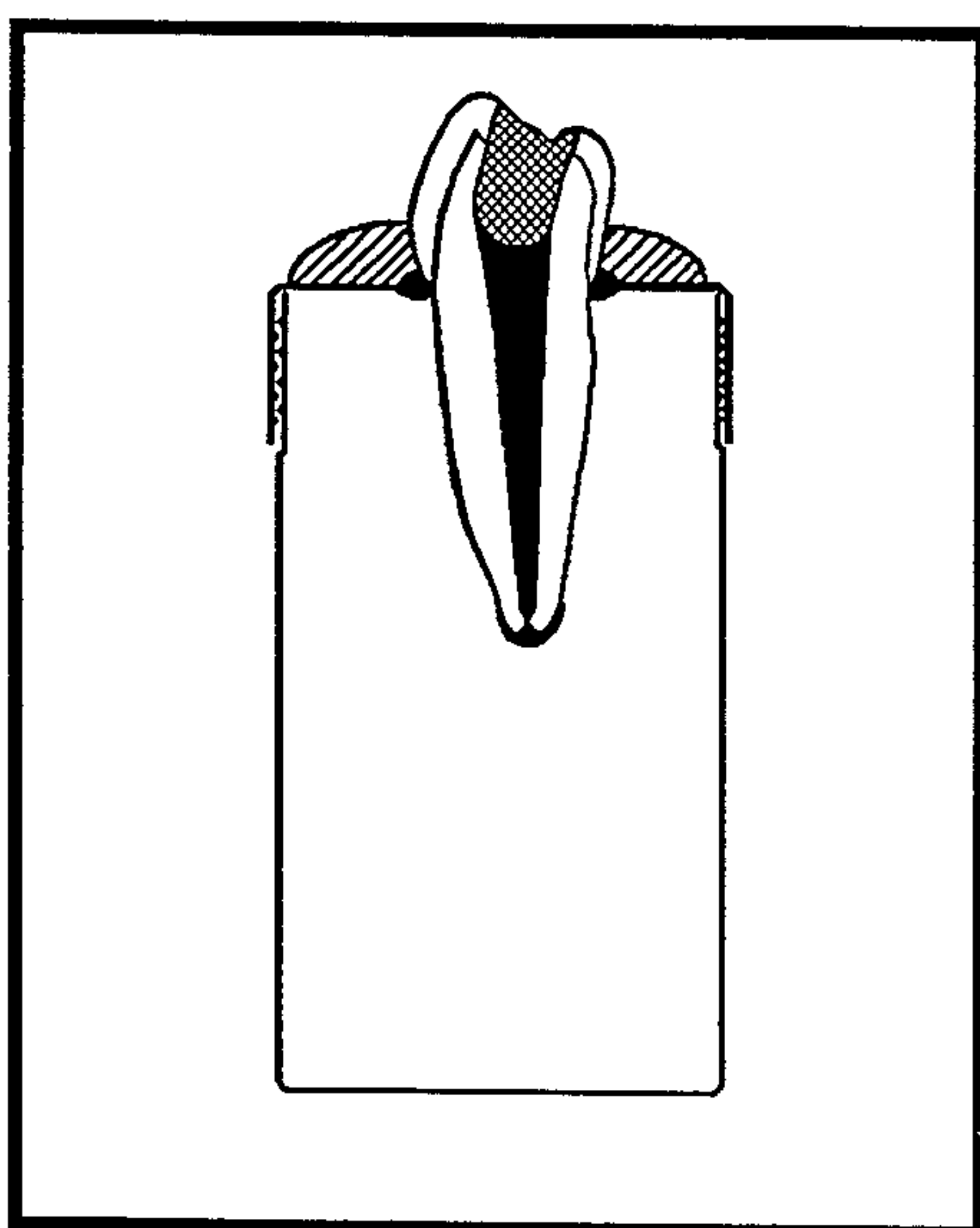


Fig. 4.4 : Obturated specimen in blank vial

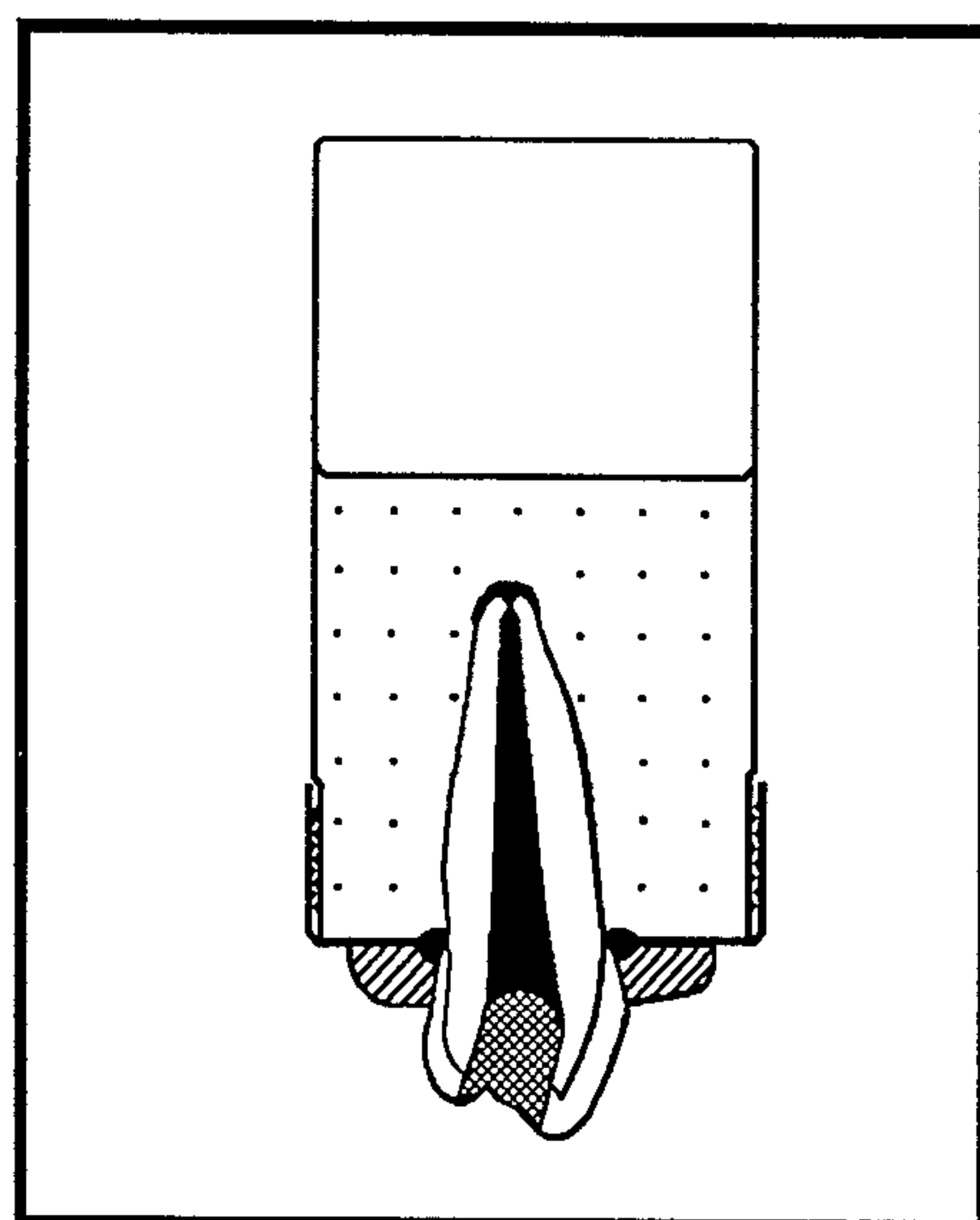


Fig. 4.5 : Numbered vial with pbs added

Eugenol was thought to be released from the ZOE root canal sealer (Proco-Sol) via the hydrolytic action of water (saline) within the root dentine. The 'liberated' eugenol was therefore available to diffuse through root dentine, down a concentration gradient, into the surrounding saline (pbs) solution. The pbs therefore functioned as a static "eluant" solution washing liberated eugenol from the exposed periodontal root surface.

Tritiated (^3H)-eugenol, released from the obturated roots, was allowed to accumulate in this eluant solution. The eluant samples were periodically collected and later analysed by LSC.

The 10 ml pbs eluation samples were collected from the twelve paired specimens (N=24) at eight logarithmically based "End-time" intervals, until termination at T = 100 days post-obturation :

T ₀	0.0	Negative control 1 min after vial inversion	
T ₁	0.03	days or 43 minutes	
T ₂	0.1	days or 2 hours 24 minutes	(144 mins)
T ₃	0.3	days or 7 hours 12 minutes	(432 mins)
T ₄	1.0	day or 24 hours	(1440 mins)
T ₅	3.0	days	(4320 mins)
T ₆	10.0	days	(14400 mins)
T ₇	30.0	days	(43200 mins)
T ₈	100.0	days or >3 months	(144000 mins)

A xylene¹ based hydrophobic solvent partitioning technique was used to separate labelled eugenol molecules from water which may have become labelled through the spontaneous dissociation of tritium atoms from parent tritiated (^3H)-eugenol molecules.

This technique permitted an accurate determination of the "eugenol only" concentration, therefore avoiding any inaccuracies that would be introduced by simultaneously determining the "non-eugenol"

1 BDH Chemicals (Analar R) - Port Fairy, Victoria

tritium atoms donated to the aqueous phase. However, a possible consequence of this dissociation was the detection and analysis of the diffusion dynamics of two molecules of dissimilar size (Pashley & Livingston, 1978), eugenol and water, under identical conditions within the same specimen (molecular radius of water = $1.97\text{\AA}$, & eugenol = $3.49\text{\AA}$ ¹)

The eluant sample from each specimen, for each (End-time) interval, was prepared as follows.

Five millilitre (5.0 ± 0.05 ml) aliquots of the original 10.0 ml pbs eluant sample were drawn-off from each collection vial using separate 2.5 ml Luer-Lok syringes² and 18 gauge needles³. An equal volume of Xylene (5.0 mls $\pm 0.1\%$)⁴, was added to each 5.0 ml pbs aliquot and immediately shaken vigorously for 20 seconds. The heterogeneous mixture was left to settle undisturbed for at least 5 mins. The process of agitation and separation was repeated three times before a 2.0 ± 0.05 ml^{1,2} sample of the xylene/solvent phase was drawn off from the supernatant and placed into a new, glass scintillation vial⁵. The latter procedure was duplicated with a second vial and solvent sample prior to the addition of Aquasol⁶ scintillant cocktail (14.0 mls $\pm 0.1\%$)³.

Due to the difficulty in preparing uncontaminated aqueous samples from the bi-phasic mixture, after having previously removed 2×2 ml solvent samples, a two millilitre (2.0 ± 0.05 ml)^{1,2} aqueous aliquot was drawn-off from the original pbs eluant solution without prior solvent extraction and then prepared for scintillation counting as before.

-
- 1 Pierens (1986) ; Uni. of Sydney - Dept Chemistry (unpublished data)
 - 2 Terumo - Tokyo, Japan
 - 3 Becton-Dickinson - Rutherford, NJ, U.S.A.
 - 4 Fortuna (Optifix Dispenser) - West Germany
 - 5 Canberra-Packard - Mt Waverley, Vic., Australia.
 - 6 Du Pont NEN Products (NEF-934)- Boston, MA, U.S.A.

The raw dpm data from these "aqueous" samples therefore represented the total tritium outflow into the eluant solution and hence required correction for the tritiated (^3H)-eugenol content, as follows :

$$[\text{raw dpm}(^3\text{H}_2\text{O}) - \text{mean dpm}(^3\text{H-E})] \times 50.6 = 10^{-12} \text{ moles tritiated water per tooth.}$$

Once scintillant had been added, each vial was shaken vigorously for 20 seconds to ensure the homogeneous distribution of components prior to beta (β^-) scintillation counting in a Packard Tri-Carb 1500¹ liquid scintillation spectrometer.

The counter was programmed (Packard Pico XTE)² to assay each sample according to the following parameters :

- * Preset-minimum counting for 10 minutes with automatic background correction, and computed corrections interpolated for variations in quench, colour and sample volume ;
- * Samples were counted against both an unquenched external standard (20 μCi ^{133}Ba) and an internal 'first vial' quenched background standard of either ;
 - (i.) 2.0 mls $\pm 1.0\%$ Xylene, or
 - (ii.) 2.0 mls $\pm 1.0\%$ p.b.s, in 14.0 mls $\pm 0.1\%$ Aquasol scintillant.
- * Data was collected in both cpm $\pm 2\sigma\%$, and dpm formats, with an index of quench and counting efficiency computed for each sample by reference to the background standards as above.

1 Packard Instrument Co.- Downers Grove, IL, U.S.A.
 2 Canberra-Packard - Mt Waverley, Vic., Australia.

4.3.1b TRITIUM CONCENTRATION ACROSS DENTINE AT PEAK OUTFLOW

Ten (10) teeth were instrumented and obturated as described before for Group A specimens (vid: 4.2.1, 4.2.2). Specimens were immersed in pbs for 24 hours, as before. Samples of powdered dentine were prepared immediately after the peak tritium outflow ($T_4 = 24$ hours), as determined during the tritium diffusion study (Group A - Part 1).

The specimens were removed from the pbs and their root surfaces gently blotted dry with paper immediately prior to sampling. Powdered dentine samples were prepared at two depths within root dentine, from the outer or periodontal (0.0-1.0 mm) and inner or mid-root (1.0-2.0 mm) levels, by shaving the root surface to an even depth with a slow-speed round bur (ISO 018) taking care not to expose the root canal filling. Periodontal depth was controlled by the axial bur-shaft dimension of the round bur whilst mid-root depth was approximated by the full diameter of the bur head.

The moist dentine powder was collected, on pre-tared sheets of aluminium foil and weighed¹ (± 0.05 mg) without drying, then immediately added to 5.0 ± 0.05 mls of pbs and the tritiated (^3H)-eugenol concentration determined by LSC after hydrophobic solvent extraction with xylene, as previously described (4.3.1a).

The raw dpm data, for tritiated (^3H)-eugenol, were expressed as picomoles eugenol per milligram of wet dentine (pmol.mg^{-1}). These concentrations were then converted to Molality based upon an extrapolation from the calculations of Hume (1984a) - that a eugenol concentration in dentine of $10 \text{ nanomoles.mg}^{-1}$ was equivalent to a 1×10^{-2} Molar eugenol solution, as below:

$$1 \text{ dpm/mg} = 10.12 \text{ pmol/mg eugenol} \quad \dots\dots\dots(1)$$

$$1 \text{ pmol.mg}^{-1} = 3.3 \times 10^{-6} \text{ M eugenol} \quad \dots\dots\dots(2)$$

$$\text{Molality} = 0.3261 (\text{dpm/mg}) \times 10^{-6} \text{ M} \quad \dots\dots\dots(3)$$

¹ Mettler (AE 160) Mettler Instrument Co - Hightstown, N.J., U.S.A.

4.3.1c QUANTIFICATION OF TRITIUM ACTIVITY CONCENTRATIONS

The tritium activity concentration of stock tritiated (^3H)-eugenol (A_t) was determined by LSC, following the obturation of Group A specimens (tritium half-life = 12.43 yrs).

A standard sample, representing a 1 in 20 dilution, was prepared by adding $50\mu\text{L} \pm 0.5\%$ of tritiated (^3H)-eugenol to $1.0 \pm 0.01\%$ (w/v) of unlabelled eugenol. This was further diluted to a 1 in 400 solution by pipetting off $50\mu\text{L} \pm 0.5\%$ of the standard sample and adding this to $1.0 \pm 0.01\%$ (w/v) of xylene solvent. The final mixture of 1.05 ml xylene/eugenol was added to Aquasol scintillant and counted as described above.

Owing to the difficulty in accurately measuring the total amount of sealer placed within each root canal at completion of obturation, only a broad approximation of recovery could be determined. Calculations were based on the A_c (moles/dpm) of each sealer mix and the final (T_8) cumulative pbs (dpm) per specimen. These were converted to a percentage figure representative of the moles of labelled eugenol recovered versus the approximated amount of sealer placed into each root canal at obturation (10 to 20 mg)¹.

¹ Stewart (1948) - J Dent Res ; 27 (1): 24-26.

4.3.2 SCANNING ELECTRON MICROSCOPY

A Joel 35-c scanning electron microscope¹ was used to assess and confirm the successful removal of the smear layer under the experimental conditions of this study. Four unpaired teeth satisfying the selection criteria (vid; 4.1) were prepared according to the experimental protocol (vid; 4.2.1), two from Group I and two from Group II.

Immediately following endodontic instrumentation, each tooth was grooved longitudinally to an approximate depth of 1.5 mm using a 0.3 mm wide high speed diamond disc² with copious water coolant. Care was taken not to perforate into the root canal space. Each specimen was fractured into two halves using side-cutting pliers, producing a total of eight specimens.

The two halves from each tooth were immediately placed into vials containing 70% Ethanol³ for 24 hours. Specimens were then transferred to 100% Ethanol (dehydrated)⁴ for 72 hours, changing the solution every 24 hours.

The dehydrated specimens were critical point dried, sputter coated with 80-90 Å of Platinum and examined utilizing the Robinson Detector⁵ and backscattered electron imaging (Boyde & Jones, 1983) with accelerating voltages set at both 10 & 25 kV, respectively.

SEM photomicrographs were taken with a Mamiya 6 x 7cm camera⁶ loaded with Ilford FP4⁷ 125 ASA film, and composite pictures constructed of the prepared root canals at low power (x10 original magnification)

-
- 1 JSM 35-c, Joel Industries - Tokyo, Japan
 - 2 Meisinger (#818/047) - West Germany
 - 3 BDH Chemicals (Analar R) - Port Fairy, Victoria
 - 4 M&B Australia Pty Ltd (Proanalys) - West Footscray, Victoria
 - 5 E.T.P. Semrar Pty Ltd - Sydney, Australia
 - 6 Mamiya Camera Co. - Tokyo, Japan
 - 7 Ilford Ltd. - Cheshire, U.K.

Representative medium power photo-micrographs (x120 and x300 original magnification) were taken of the apical, mid-root and coronal regions of each tooth, for each preparation technique. The photo-micrographs were processed and qualitatively examined for the presence or absence of a smear layer.

Photomicrographs from Group II specimens (smear layer removed and tubule orifices exposed) were also qualitatively examined for variations in dentinal tubule density and size throughout the length of the canal.

4.4. Calculation of the Lateral Surface Area available for Diffusion.

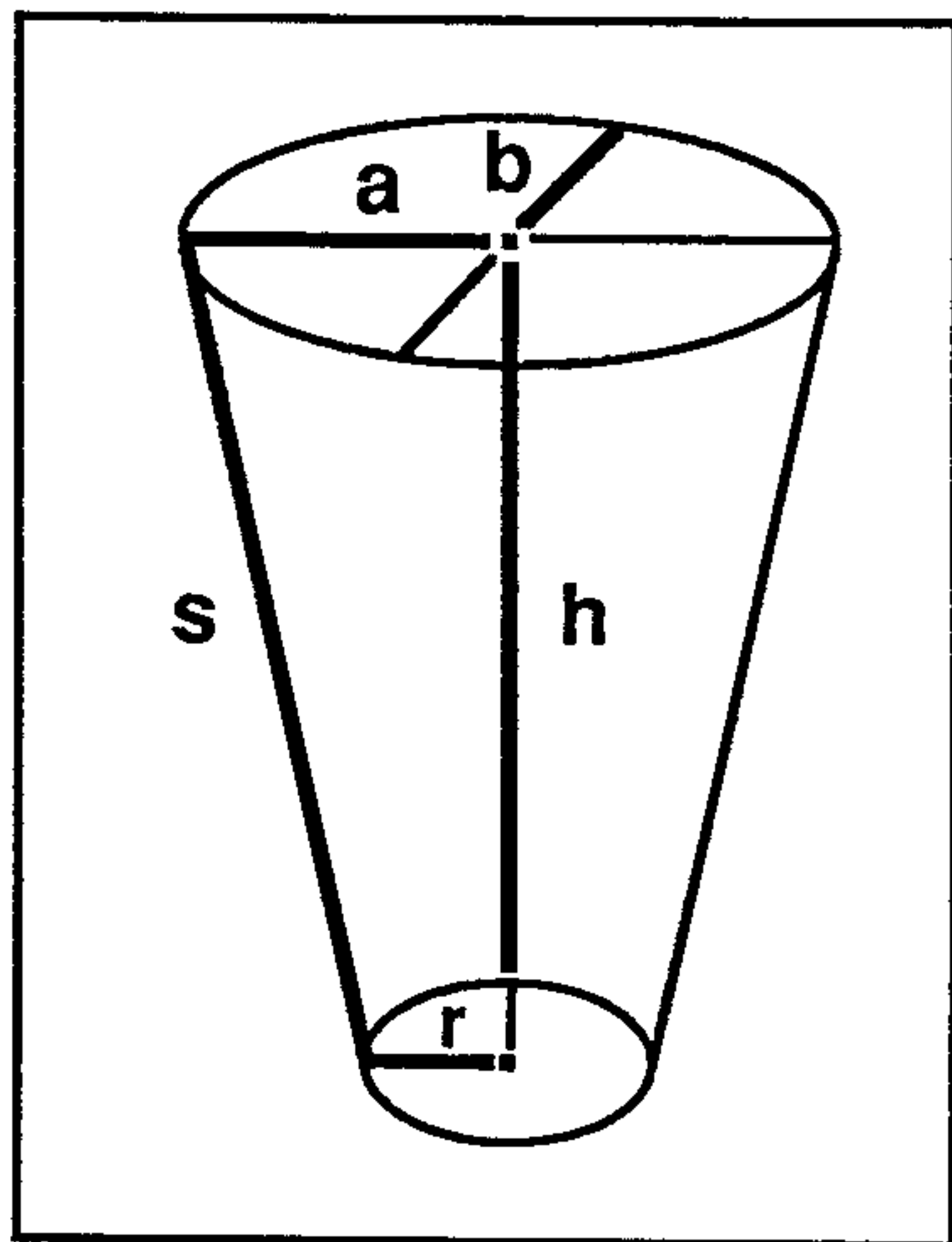
At the completion of study each Group A tooth was sectioned transversely, at its level of emergence from the vial cap, with a high speed tungsten carbide bur under water spray. Each canal was then examined, under a linear vernier microscope¹ at x30 magnification, to measure (± 0.01 mm) its axial length, major and minor coronal diameters. The apical radius was taken as half the tip diameter of the M.A.F. used during instrumentation.

It was assumed that the shape of an instrumented canal was most closely represented by a 'frustum' - a truncated cone, with an elliptical base and a circular apex. The following formula² was applied to calculate the internal lateral surface area for each canal.

Lateral area of an Elliptical frustum (S_e) equals half the sum of the perimeters of each of its bases multiplied by the height of slant.

$$S_e = \frac{1}{2}(P_1 + P_2).h \quad \text{that is,}$$

$$= \frac{1}{2} \{2\pi.[r + \sqrt{\frac{1}{2}(a^2 + b^2)}]\} \times \sqrt{[(a-r)^2 + h^2]}$$



Measurements were taken of :

h = length of root available for diffusion
(distance from CEJ wax to Apical seal)

r = the radius at the apical seat
($\frac{1}{2}$ x MAF size)

a = major axis of the coronal ellipse
($\frac{1}{2}$ x B-L canal width at the CEJ wax)

b = minor axis of the coronal ellipse
($\frac{1}{2}$ x M-D canal width at the CEJ wax)

Teeth were subsequently sub-divided into three groups : ≤ 40 mm², 41 to 60 mm², and ≥ 61 mm²; for correlation between variation(s) of the mean rate(s) of tritiated (³H)-eugenol diffusion and the internal lateral surface area available for diffusion.

1 Griffin & George - London, U.K.

2 CRC Standard Mathematical Tables (1981) - Beyers: 26th ed.; 127-128.

4.5 STATISTICAL ANALYSIS

Paired, two-tailed Student T-Tests (Phillips, 1978) were applied to the mean release rate data for tritiated-eugenol and tritiated-water, from Group I and Group II specimens, at each of eight end-time intervals.

The parameters for significance were as follows :

α - Level = 0.05

df = 18, (two-tailed analysis)

$\{H_0 : \mu_1 \neq \mu_2\} ; \{H_1 : \mu_1 = \mu_2\}$

if the calculated t-score < 2.101, then accept H_0

i.e. "a difference could not be statistically guaranteed between the mean(s) of Group I and II under the present conditions of study."

Two factor ANOVA Tests (α -level = 0.01) were applied to the mean tritiated-eugenol and tritiated-water rate release data for pairs of specimens re-grouped according to :

- a) history - sex, age, delay from extraction to instrumentation, and
- b) preparation - volume of irrigant, M.A.F. size, lateral surface area.

Any significant relations were to be further tested by applying the Scheffé test (Phillips, 1978).

CHAPTER 5

RESULTS AND STATISTICAL ANALYSES

5.1.1 SPECIMEN DATA

Over 200 teeth were collected prior to study but only 38 of these satisfied the selection criteria. Most teeth were rejected owing to their contralateral 'homologue' not being collected at the subsequent extraction. Several otherwise suitable pairs of teeth were also discarded due to incomplete documentation or the complicated extraction of either tooth within the pair.

All 38 teeth used in both parts of this study were from individuals with a life time exposure to fluoridated water in the Sydney Metropolitan Region. All teeth were collected, as specified, from patients about to commence orthodontic therapy.

The demographic particulars for Group A specimens (N=24) were as follows (App. 1.1): 42% were male and 58% female; with one pair aged 12-14 years, nine pairs 14-16 yrs, and two pairs 16-18 yrs. The delay between extraction of each tooth within a pair ranged from 0-8 days. All teeth were extirpated within seven days of extraction. The delay between extraction and obturation ranged from 15-141 days with five pairs <56 days and seven pairs >56 days.

5.1.2 PREPARATION DATA

The twenty experimental teeth, used during the dentine diffusion study, were instrumented with the following particulars (App. 1.2).

Apical stops were prepared to a MAF size #45 in six pairs, #35 in three pairs and size #30 in one pair. The volumes of irrigant used during canal preparation were 15 mls in three pairs, 20 mls in five pairs, and 25 mls in two pairs and did not include the experimental final flush volume (20 mls).

There were minor variations in working length, within pairs, due primarily to differences in cuspal reduction at access preparation. Working lengths ranged from 18.5 mm to 23.0 mm. Each pair of teeth was instrumented and obturated on the same day with a maximum delay between teeth of three hours.

Calculation of the lateral internal surface areas (LSA) available within each canal determined that LSA for one pair was $\leq 40 \text{ mm}^2$, five pairs were $40\text{-}60 \text{ mm}^2$, and four pairs were $\geq 60 \text{ mm}^2$ (App. 1.3).

The mean ($\pm$ sem) LSA for Group I - smear present specimens (N=10), was $58.42 \pm 3.04 \text{ mm}^2$ and for Group II - smear removed specimens (N=10), was $58.65 \pm 3.31 \text{ mm}^2$ whilst the pooled mean LSA (N=20) was $58.58 \pm 2.19 \text{ mm}^2$. The mean difference in LSA between teeth, within a homologous pair (N=10 pairs), was $4.15 (\pm 0.79) \text{ mm}^2$ or about 7% (Table : 5.1 below).

Lateral Internal Surface Area			
Specimens	mean (mm^2)	$\pm$ sem	number
Group I	58.42	3.04	N = 10
Group II	58.65	3.31	N = 10
pooled data	58.58	2.19	N = 20
diff within pairs*	4.15	0.79	N = 10 pr

Table : 5.1 - Summary of Lateral Internal Surface Areas for Part 1 specimens

5.2 LIQUID SCINTILLATION DATA

Tritiated pbs samples (without solvent extraction) were consistently counted with 35-36% efficiency, whilst solvent sample (tritiated-eugenol) counting efficiency ranged between 55-61%. The counting accuracy ($\pm 2\sigma\%$) of the protocol selected was consistent for all specimens during each end-time interval.

5.2.1 PART A : Tritium Outflow

The mean rate release data, for Groups I and II, is summarized in tables 5.2.1 & 5.2.2 below, and presented in full in Appendix 1.1 (tables A1.1a through A1.2e).

These data showed no statistically significant difference(s) between the mean release rates of Group I or II specimens, for either tritiated-eugenol ($P > 0.35$) or tritiated-water ($P > 0.15$), at any end-time interval throughout the duration of study.

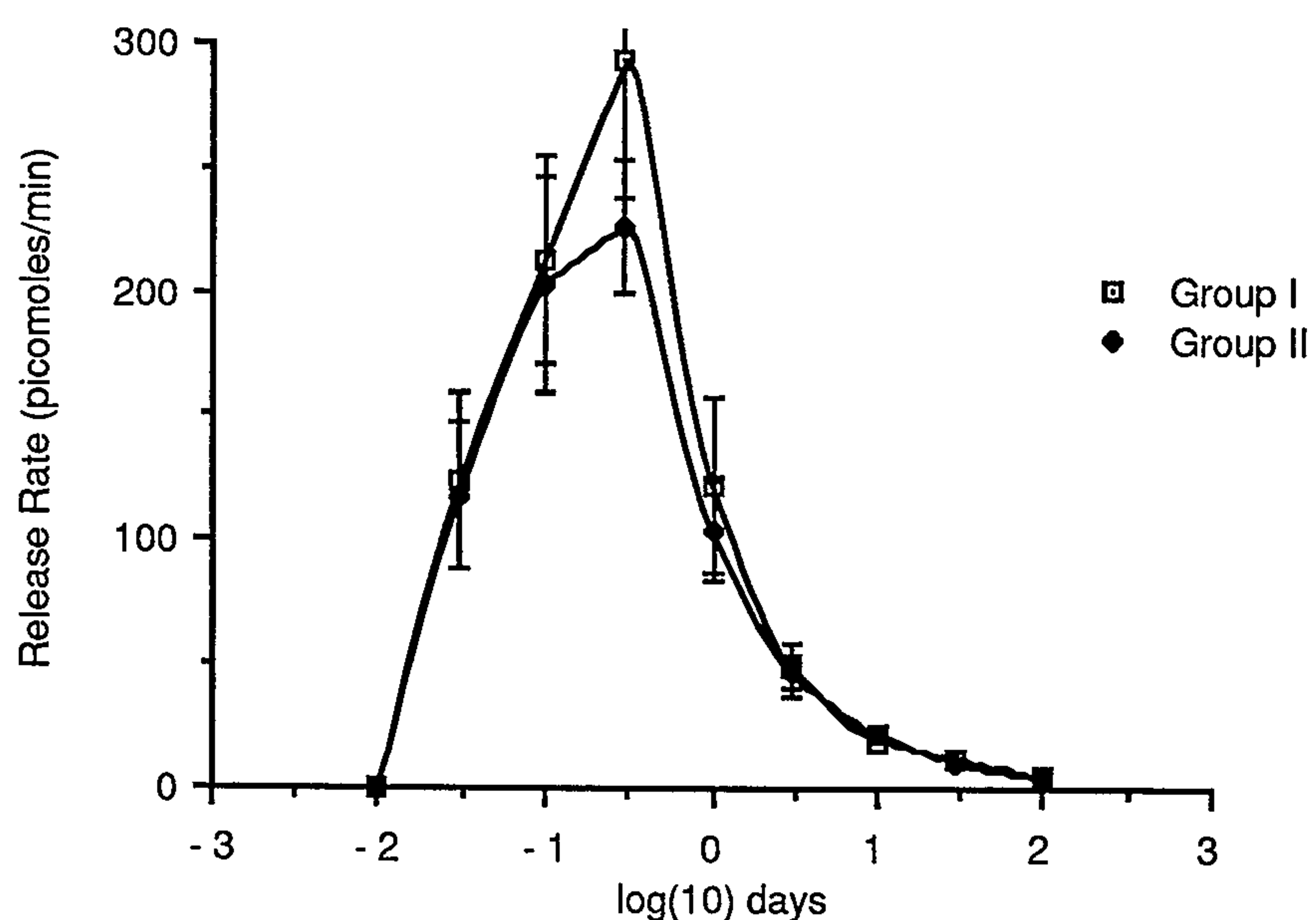
Table: 5.2.1- RELEASE RATE OF EUGENOL (10^{-12} M.min⁻¹ of ³H-E)

GROUP I - MILTONS/Smearcd		GROUP II - EDTAC/Smear Removed		
End-Time	Mean(rate) $\pm$ sem	Mean(rate) $\pm$ sem	t-score	Significance
T ₀ =1 min	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.0000	> 0.9
T ₁ =43	15.87 $\pm$ 8.25	24.11 $\pm$ 11.95	-0.5674	> 0.6
T ₂ =144	8.16 $\pm$ 2.40	7.95 $\pm$ 2.40	-0.1568	> 0.85
T ₃ =432	14.37 $\pm$ 4.56	10.52 $\pm$ 2.77	0.7208	> 0.45
T ₄ =1440	9.49 $\pm$ 4.40	9.68 $\pm$ 3.73	-0.0821	> 0.9
T ₅ =4320	8.48 $\pm$ 3.42	7.46 $\pm$ 2.22	0.2480	> 0.8
T ₆ =14400	4.07 $\pm$ 0.73	4.90 $\pm$ 1.07	0.6668	> 0.5
T ₇ =43200	3.74 $\pm$ 0.98	3.34 $\pm$ 0.89	0.2974	> 0.75
T ₈ =144000	1.89 $\pm$ 0.29	1.49 $\pm$ 0.33	0.8944	>0.35

Table 5.2.2 - RELEASE RATE OF TRITIATED WATER (10^{-12} M min^{-1} of ^3H -Water)

GROUP I - MILTONS/Smeared		GROUP II - EDTAC/Smear Removed		
End-Time	Mean(rate) $\pm$ sem	Mean(rate) $\pm$ sem	t-score	Significance
T ₀ =1 min	0.00 $\pm$ 0.00	146.72 $\pm$ 146.72	-1.0000	>0.3
T ₁ =43	124.19 $\pm$ 35.29	118.14 $\pm$ 29.71	0.1294	>0.9
T ₂ =144	213.58 $\pm$ 41.86	203.13 $\pm$ 44.03	0.1728	>0.9
T ₃ =432	293.13 $\pm$ 55.72	226.32 $\pm$ 27.24	1.0777	>0.25
T ₄ =1440	122.23 $\pm$ 34.90	103.47 $\pm$ 20.24	0.4658	>0.65
T ₅ =4320	48.17 $\pm$ 11.32	46.62 $\pm$ 7.19	0.1096	>0.9
T ₆ =14400	18.81 $\pm$ 4.20	21.66 $\pm$ 2.83	-0.5580	>0.55
T ₇ =43200	11.83 $\pm$ 1.81	10.37 $\pm$ 1.44	0.6568	>0.5
T ₈ =144000	4.51 $\pm$ 0.49	3.47 $\pm$ 0.41	1.5704	>0.15

The overall release phenomenon displayed parallel time/rate characteristics for the diffusion of both tritiated eugenol (^3H -E) and tritiated water (^3H -water). In general terms, the tritium outflow increased rapidly to an initial peak, during the first day, which was followed by a progressive decline until the conclusion of study at the 'end time' ; T₈ = 100 days (Figs: 5.1 and 5.2).

Fig : 5.1 Release Rate of tritiated (^3H)-water

Although very similar, the release characteristics of each molecule were different in two respects. Firstly, a distinct depression was observed in ^3H -E outflow, at $T_2 = 144$ mins (2 hrs 24 mins), which occurred immediately after the moment of peak release, $T_1 = 43$ mins (Fig : 5.2). The peak rate of ^3H -E outflow ($T_1 = 43$ mins) also occurred earlier than that for peak ^3H -water release which occurred at $T_3 = 432$ mins or about 8 hrs post-obturation (Fig : 5.1).

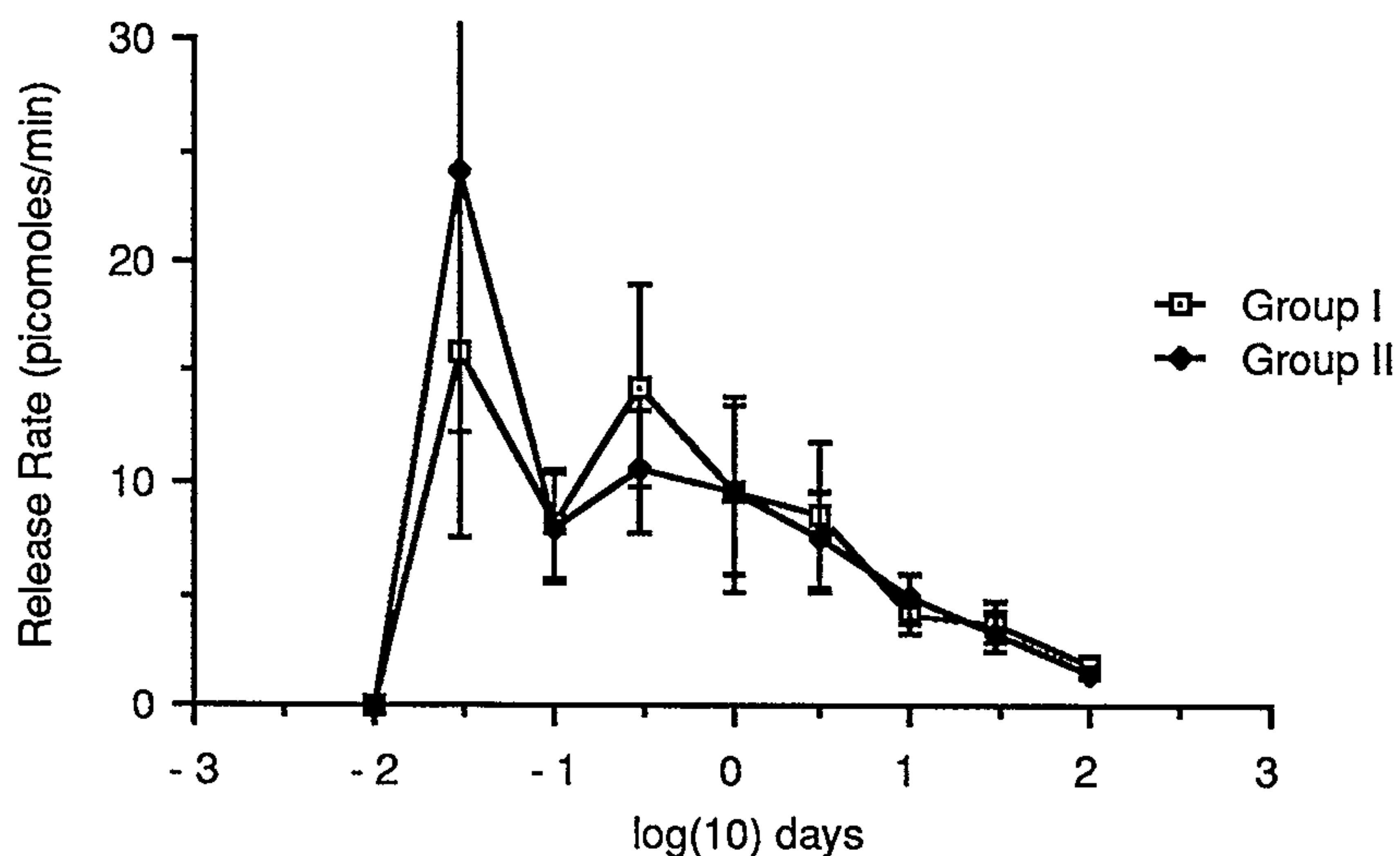


Fig : 5.2 Release Rate of Tritiated (^3H)-Eugenol

Secondly, the absolute tritium (^3H) concentrations detected in the aqueous phase (i.e. ^3H -water) were markedly greater than those detected within the solvent phase (i.e. ^3H -E) throughout the duration of the current study (100 days).

This is graphically presented for both Group I and Group II specimens in Figs : 5.3 and 5.4 (see over).

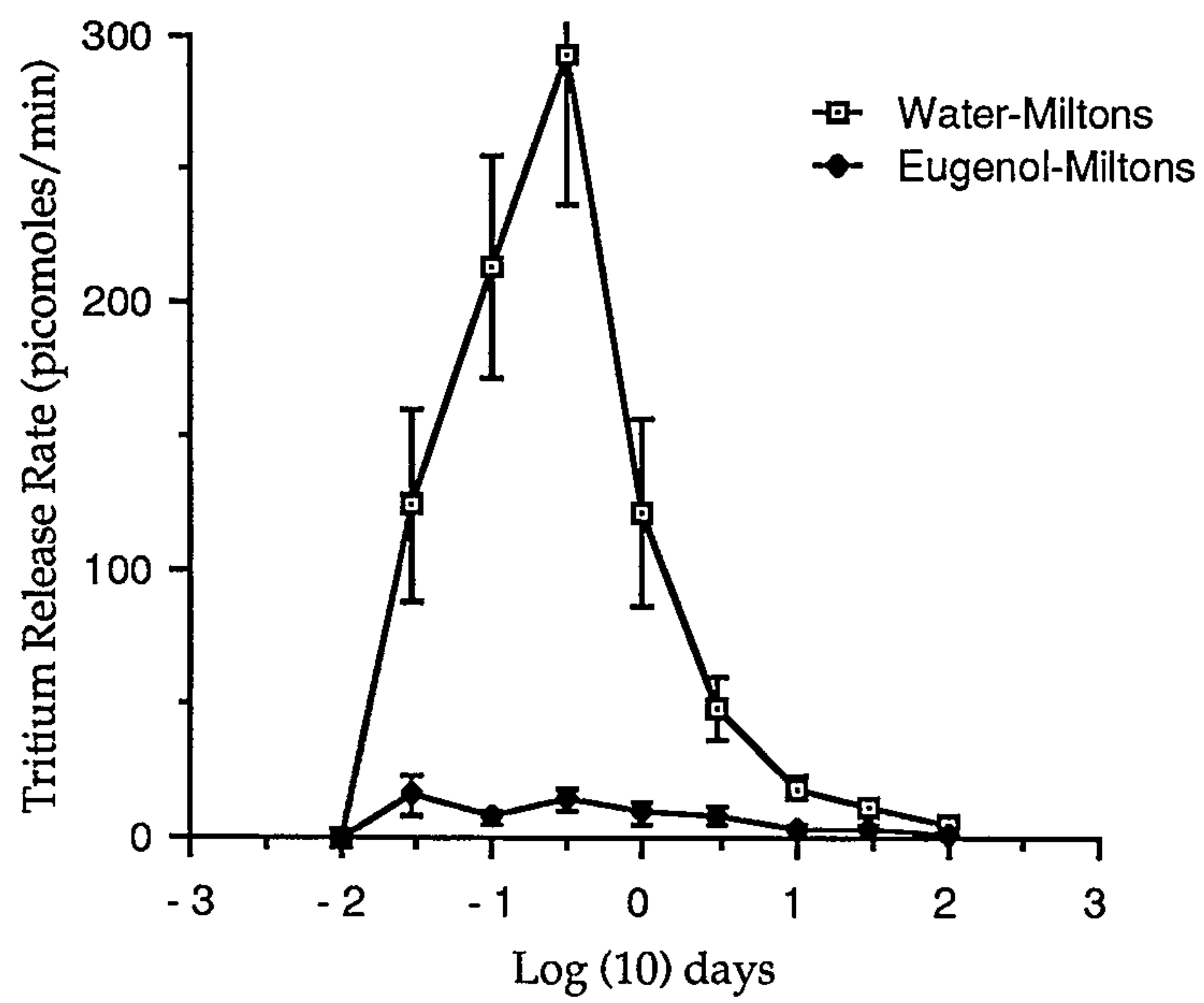


Fig : 5.3 Tritium Outflow for Group I (smear present) specimens

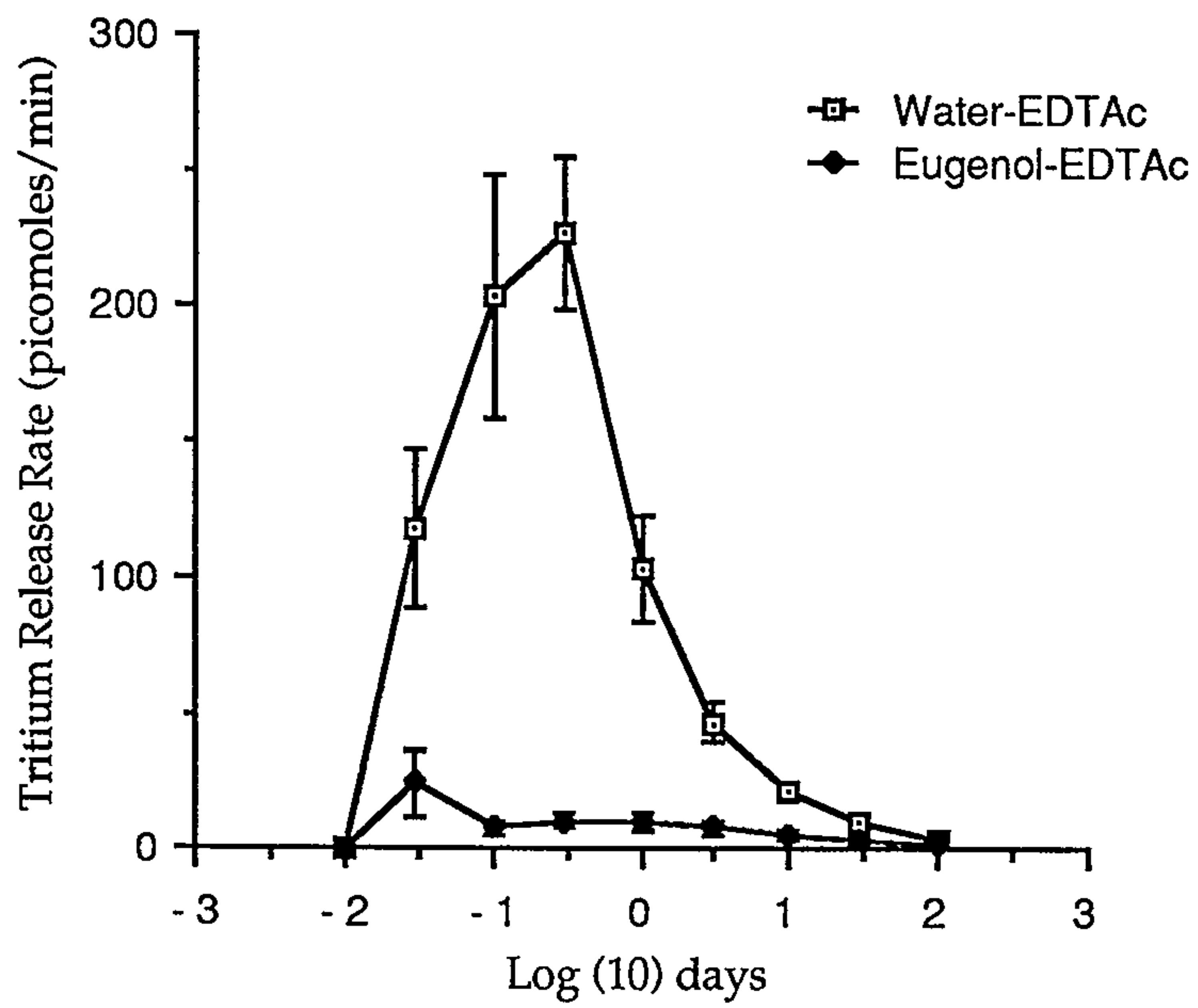


Fig : 5.4 Tritium Outflow for Group II (smear removed) specimens

Analysis of the comparative rates of tritium release, for both Group I and Group II specimens, confirmed that the ratios of release rate [^3H -water : ^3H -E] also varied synchronously with the general release phenomenon (Fig : 5.5).

The rate of change in these ratios did not appear to follow any predictable pattern, i.e. neither linear nor logarithmic, but was graphically interpolated by a complex polynomial regression curve,

$$y = -0.64 + 2.42x + 13.57x^2 - 6.57x^3 + 1.02x^4 - 0.05x^5 \quad (\text{where } R = 0.96)$$

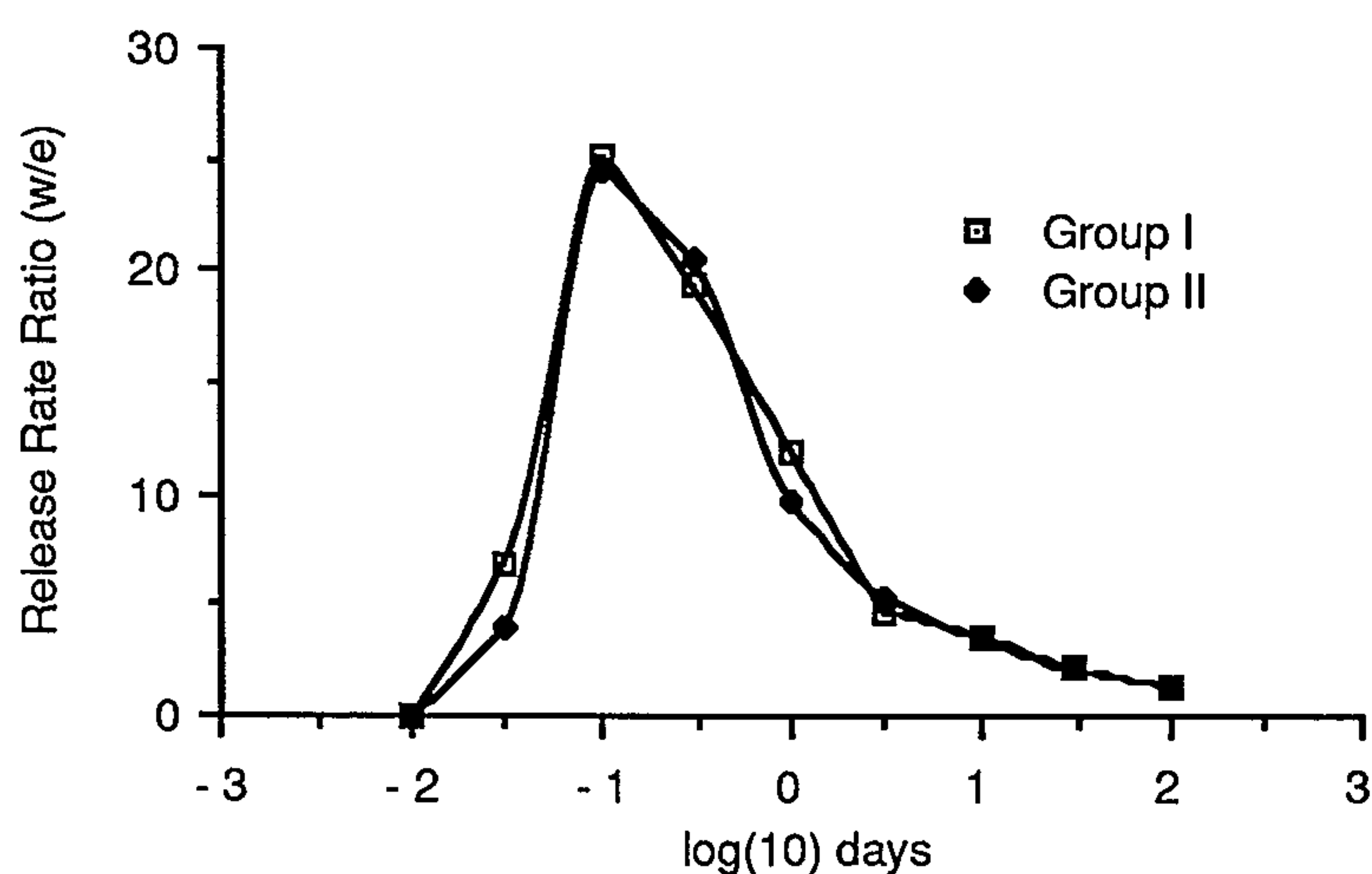


Fig : 5.5 Mean Release Rate Ratios for water/eugenol

It was also of peculiar interest to note that the mean differences in rate release data between heterologous specimens, from different patients but the same experimental grouping, were markedly greater than those between groups, from the same patient (e.g. Fig : 5.6). This was true for both tritiated-water and tritiated-eugenol, at all end-time intervals until $T_8 = 100$ days.

(Eugenol only data shown over; Fig : 5.6)

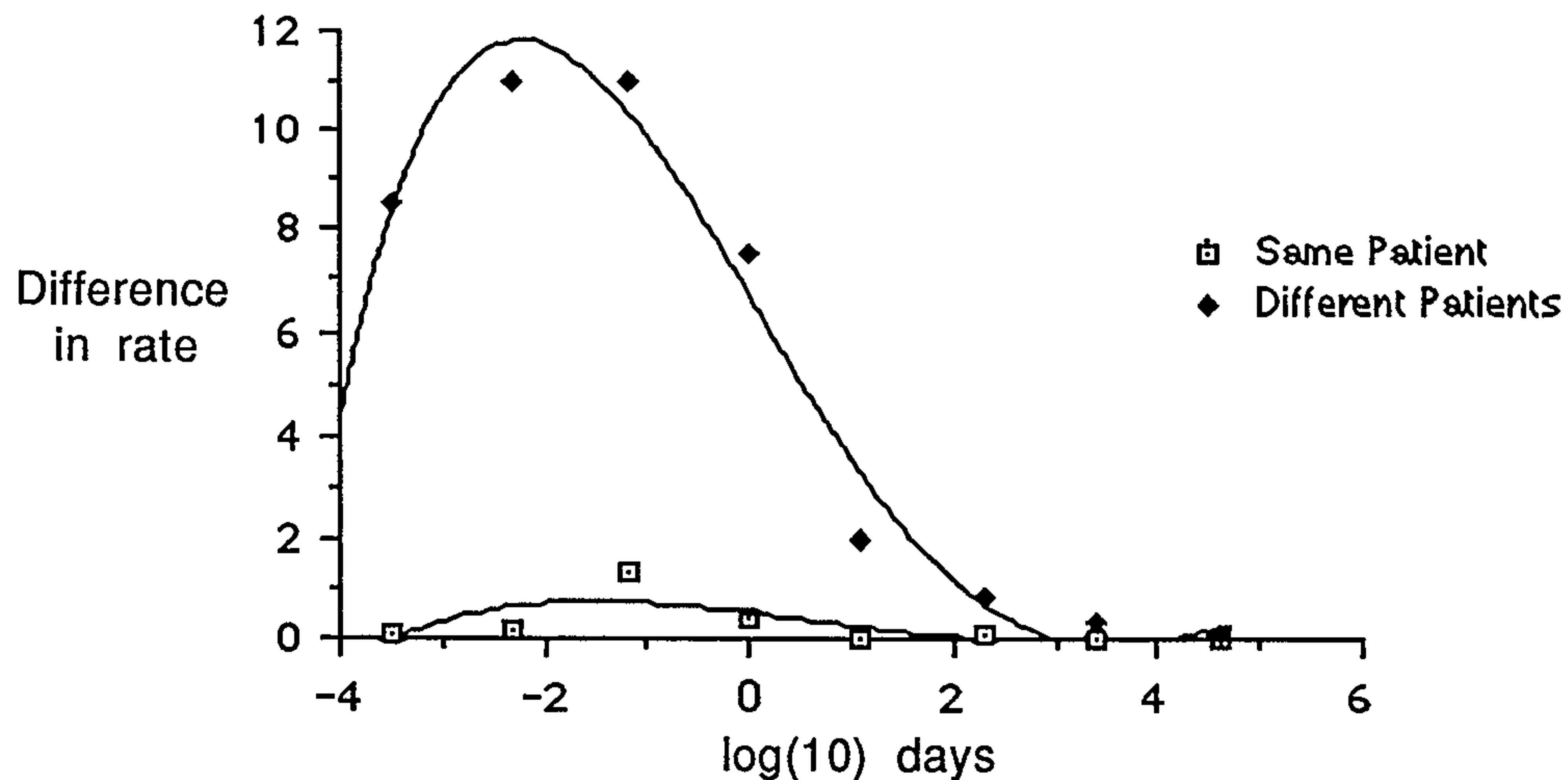


Fig : 5.6 Mean Difference(s) in Eugenol Release Rate

Further statistical analysis, using multiple two factor ANOVA, demonstrated there were no 'apparent' differences in mean release rates immediately following peak outflow which could be attributed to sub-groups based upon sex, age, post-extraction interval, irrigant volume, M.A.F. size or lateral internal surface area. Unfortunately, the sample sizes in the present study were too small to draw any relevant conclusions. However, there was a weak tendency for the absolute peak $^3\text{H-E}$ release to vary proportionately with increasing lateral internal surface area ($p > 0.1$) as graphically demonstrated below, Fig. 5.7 and in Table A2.2f.

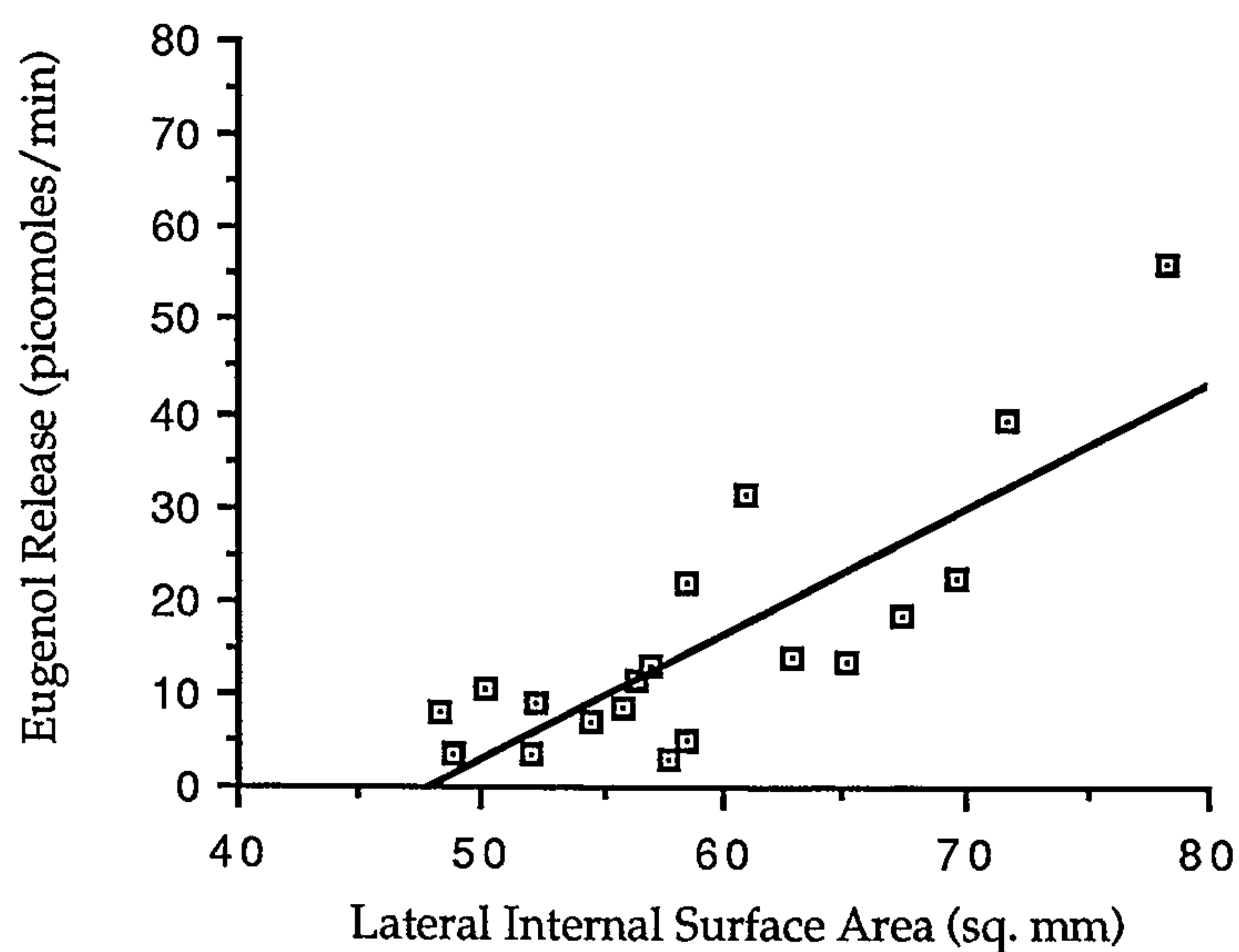


Fig : 5.7 Surface Area vs Absolute Peak $^3\text{H-E}$ Release (Pooled data)

5.2.2 Peak Eugenol Concentration in Root Dentine

The peak tritiated (³H)-eugenol concentration in root dentine varied consistently with the depth from which the samples were taken, with higher concentrations closest to the root canal or pulp, and lower concentrations near to the periodontal root surface as below; (Tables : 5.2.3 and 5.2.4).

The mean peak tritiated (³H)-eugenol concentrations for each group at each depth sampled showed no statistically significant difference(s) Therefore, the data from each group were pooled at each depth and the concentration gradient evaluated graphically (Fig ; 5.8).

Peak Eugenol concentration in Mid-root dentine at T ₄ = 24 hrs			
	Group I	Group II	Pooled data
mean±sem	3.93 ±0.95	3.62 ±0.96	3.65 ±0.64
Peak Eugenol concentration in Periodontal dentine at T ₄ = 24 hrs			
	Group I	Group II	Pooled data
mean±sem	1.74 ±0.38	1.44 ±0.31	1.71 ±0.23

Table 5.2.3 - Eugenol Concentrations in Root Dentine (picomoles/mg)

where Group I are smear present specimens (Miltos)
 Group II are smear removed specimens (EDTAC)

Molar Eugenol concentration in Mid-root Dentine (at T ₄ = 24 hrs)			
	Group I	Group II	Pooled data
mean±sem	12.8 ±3.10 μM	11.9 ±3.13 μM	12.1 ±2.09 μM
Molar Eugenol concentration in Periodontal Dentine (at T ₄ = 24 hrs)			
	Group I	Group II	Pooled data
mean±sem	5.67 ±1.24 μM	4.76 ±1.01 μM	5.22 ±0.75 μM

Table 5.2.4 - Eugenol Molality in Root Dentine

where Group I are smear present specimens (Miltos)
 Group II are smear removed specimens (EDTAC)

If one assumes that the mean peak concentration gradient between pulpal/middle dentine and periodontal dentine was a simple linear relationship, then the slope of the gradient was about [2.52 : 1].

However, graphical interpolation suggests a complex relationship which can best be defined by a logarithmic equation ;

$y = 1.007 \log_e + 5x^{-11.4662}$ (R = 0.95) and depicted below in Fig : 5.8

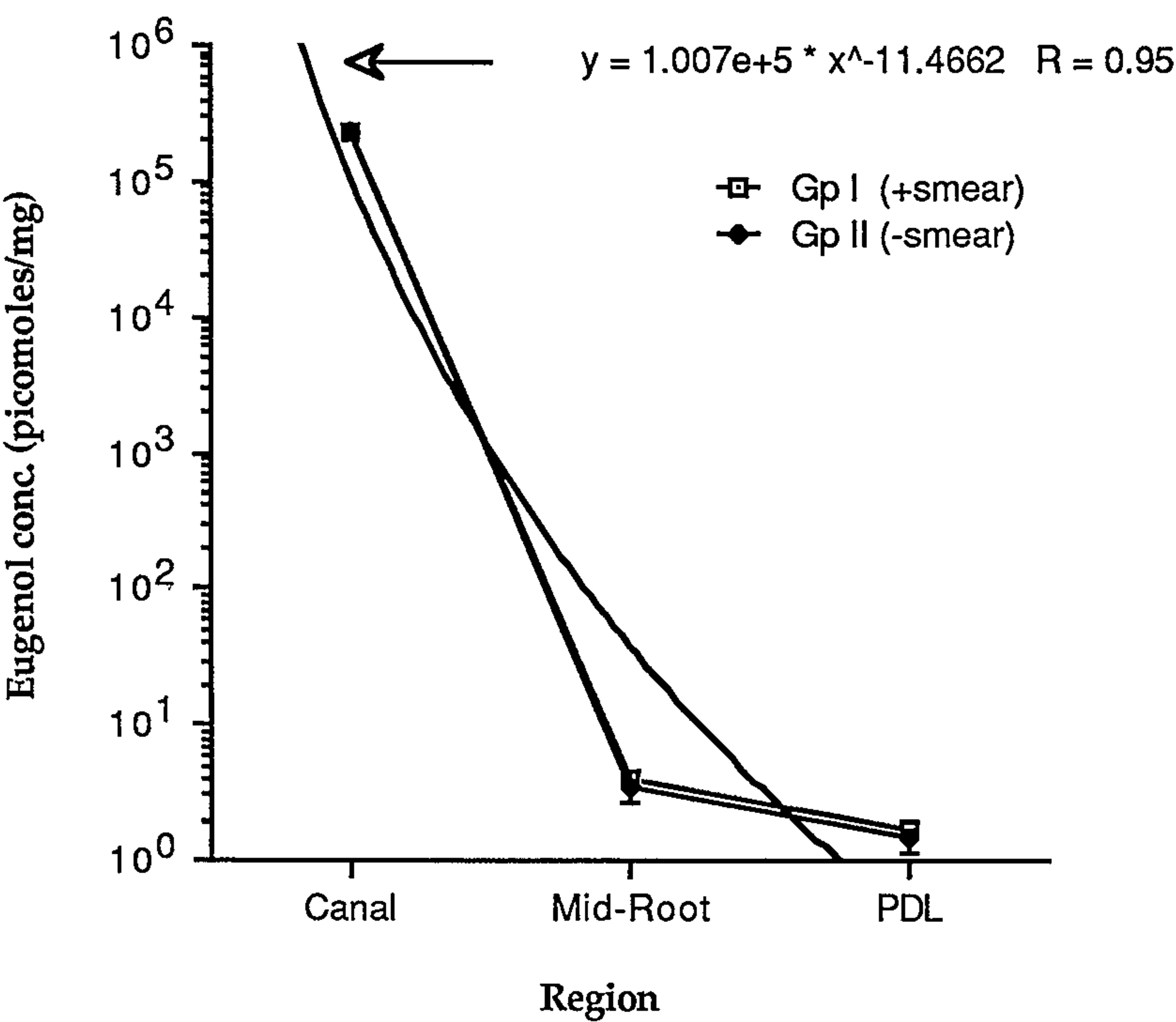


Fig : 5.8 - Eugenol Concentration Gradient following Peak Outflow

5.2.3 Specific Activity determinations for tritium

The labelled stock solution of tritiated (^3H)-eugenol was found to have a tritium activity (A_t) = 1.6×10^6 dpm (October 1987). This was equivalent to an $A_0 = 164.65 \times 10^7$ Bqmole $^{-1}$ or 44.5×10^{-3} Cimole $^{-1}$.

Each disintegration (dpm) could therefore be interpreted such that ;
 $1 \text{ dpm} = 10.12 \times 10^{-12}$ moles of stock tritiated-eugenol liquid.

The standardized mix of Procosol sealer was analysed, by LSC, to possess an Activity concentration (A_c) = 23×10^6 dpm per mix.

This was the equivalent of 39.5×10^4 Bq or $10.68 \mu\text{Ci}$ per 0.218 gram mix of sealer. Approximately 10 to 20 mg of sealer were placed into each tooth at obturation which represented 0.233×10^{-4} moles of eugenol or an $A_c = 10^2$ Bq ($10^{-2} \mu\text{Ci}$) per obturated canal.

5.2 SEM RESULTS

Two distinctly different and contrasting surface appearances were seen in the photomicrographs of Group I and Group II specimens.

A definite and complete smear layer was observed throughout the instrumented root canal of Group I specimens, on both low magnification (Fig: 5.9) and higher magnification (Figs: 5.11 to 5.17) photomicrographs.

In contrast, photomicrographs from Group II specimens (Figs: 5.10, and 5.18 to 5.24) consistently showed the total removal of the smear layer, throughout the entire root canal. Removal of the smear layer revealed the highly fibrillar nature of the predominantly organic pre-dentine and exposed the underlying tubule orifices (Fig: 5.10).

Several subjective observations were made of the exposed "pulpal" dentine surface, from Group II photomicrographs, as revealed by the experimental removal of the smear layer. No quantification of these observations were attempted as the specimen preparation and examination techniques were not designed to substantiate such conclusions.

The subjective observations included ;

- that the number of dentinal tubules per unit area (density) decreased from the coronal to apical regions.
- the apparent diameter of each tubule decreased, similarly, in an apical direction.
- the amount of inter-tubular dentine, and hence the relative inter-tubular distance, increased toward the apical region.
- the relative amount of exposed pre-dentine, within each tubule orifice, decreased as the apex was approached.

The following representative photomicrographs depict the surface topography of Group I and Group II specimens at high magnification and confirm the successful removal of the smear layer via the experimental irrigation technique (Yamada et al, 1983) used to prepare Group II specimens.

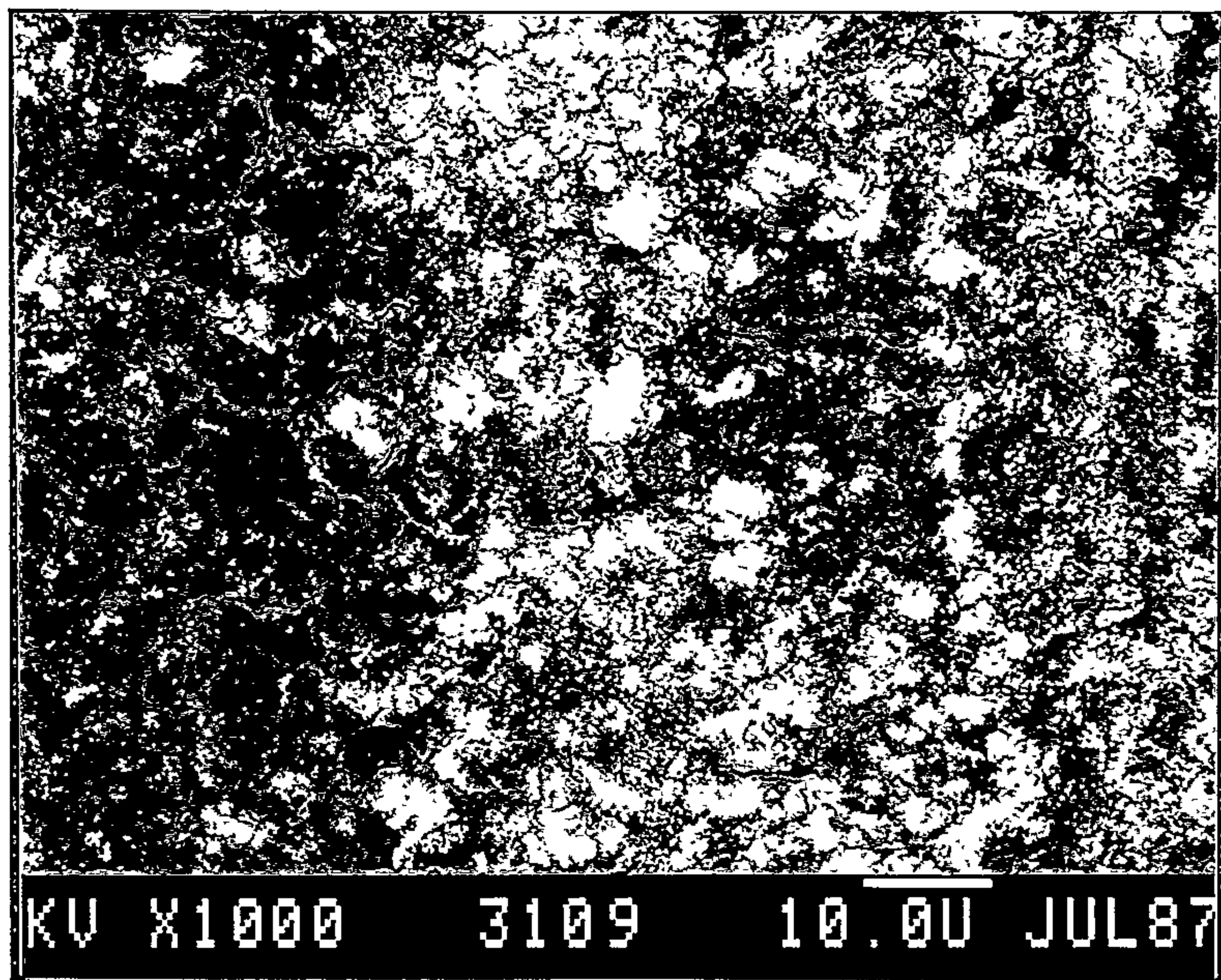


Fig : 5.9 - Group I Smear Layer Present (x1000 orig. mag.)

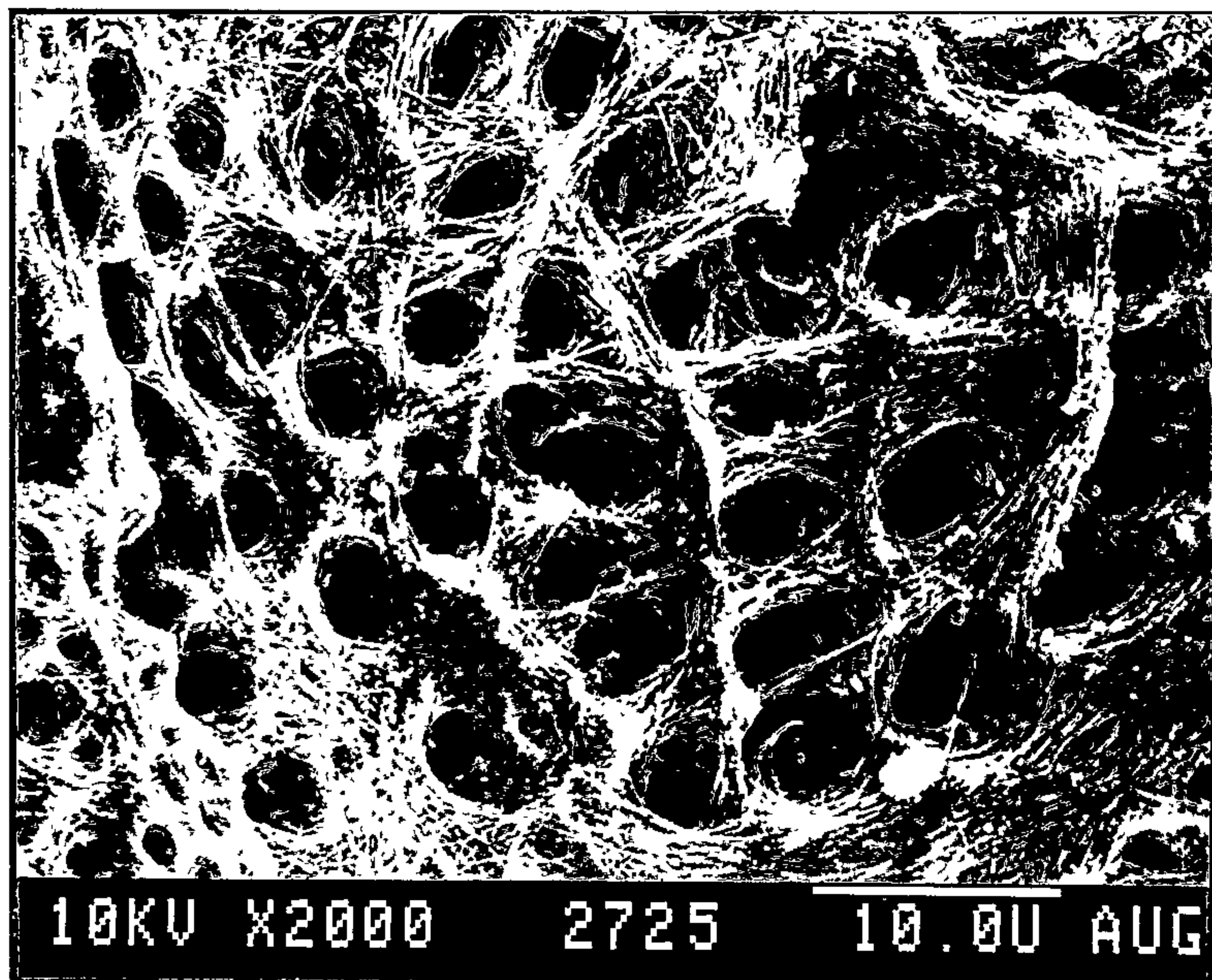


Fig : 5.10 - Group II Smear Layer Removed (x2000 orig. mag.)

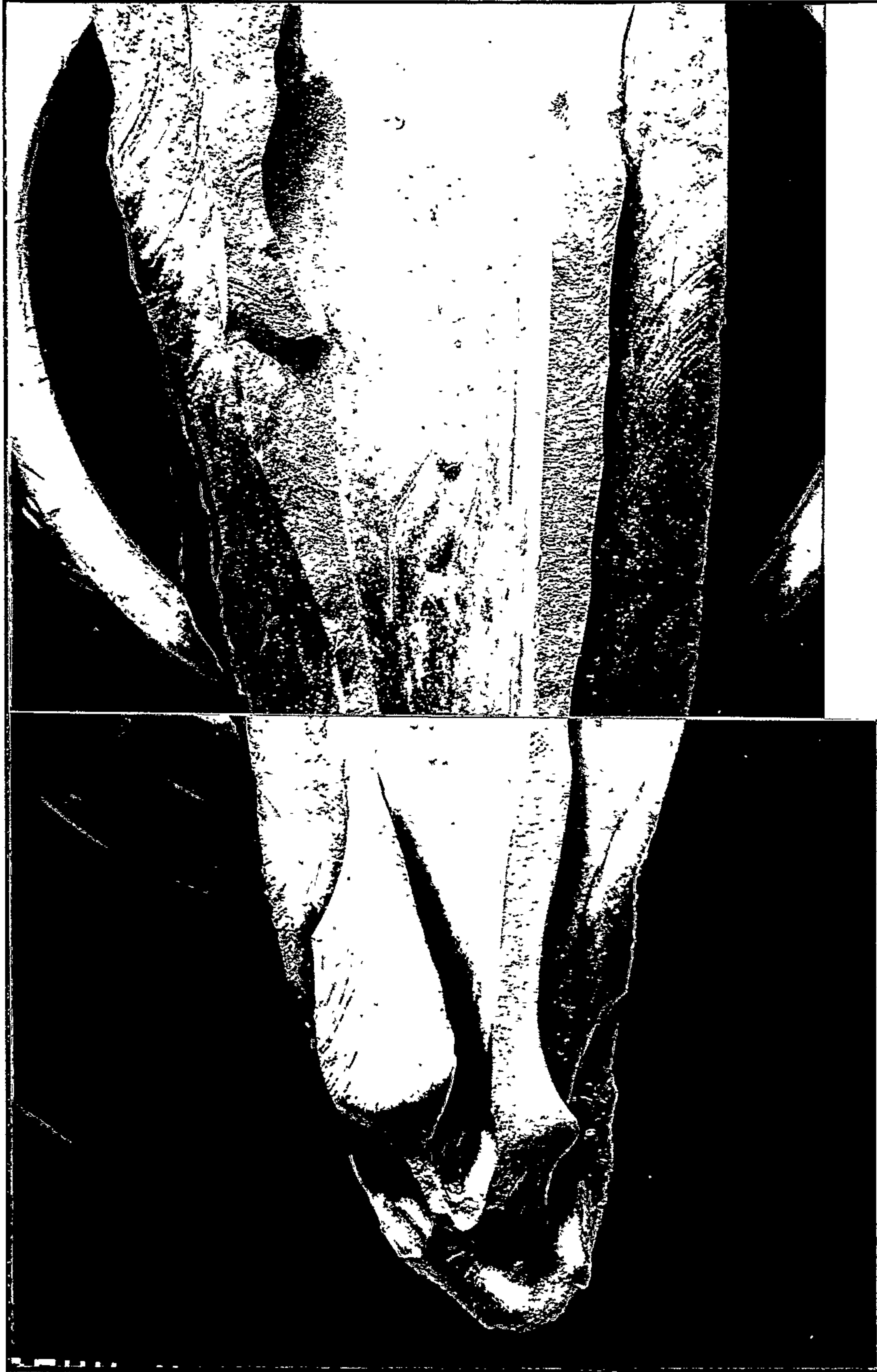


Fig: 5.11 - Group I Smear Layer Present (x10 original magnification).

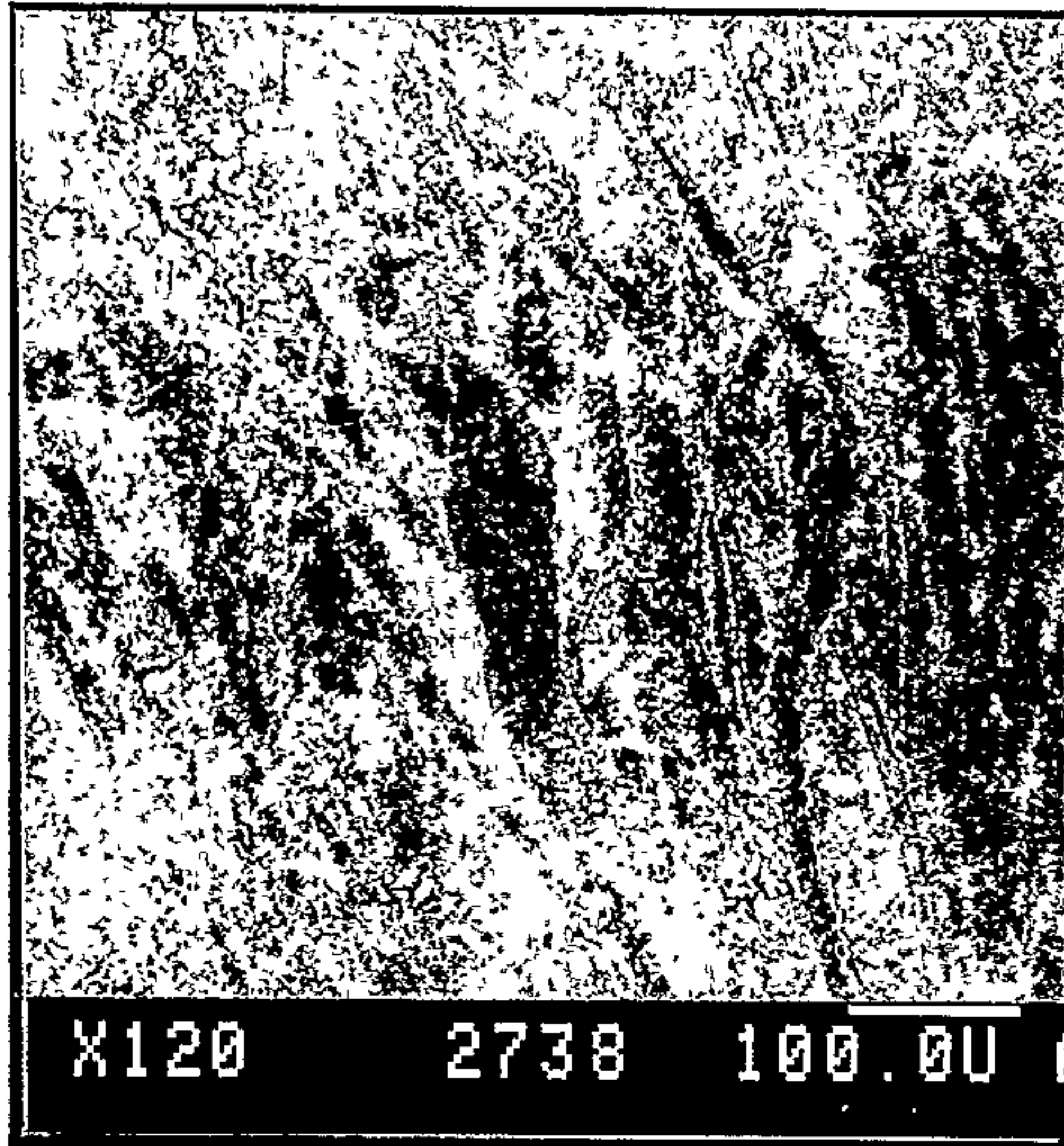


Fig: 5.12 Group I Coronal Third (x120 orig)



Fig: 5.13 Group I Coronal Third (x300 orig)

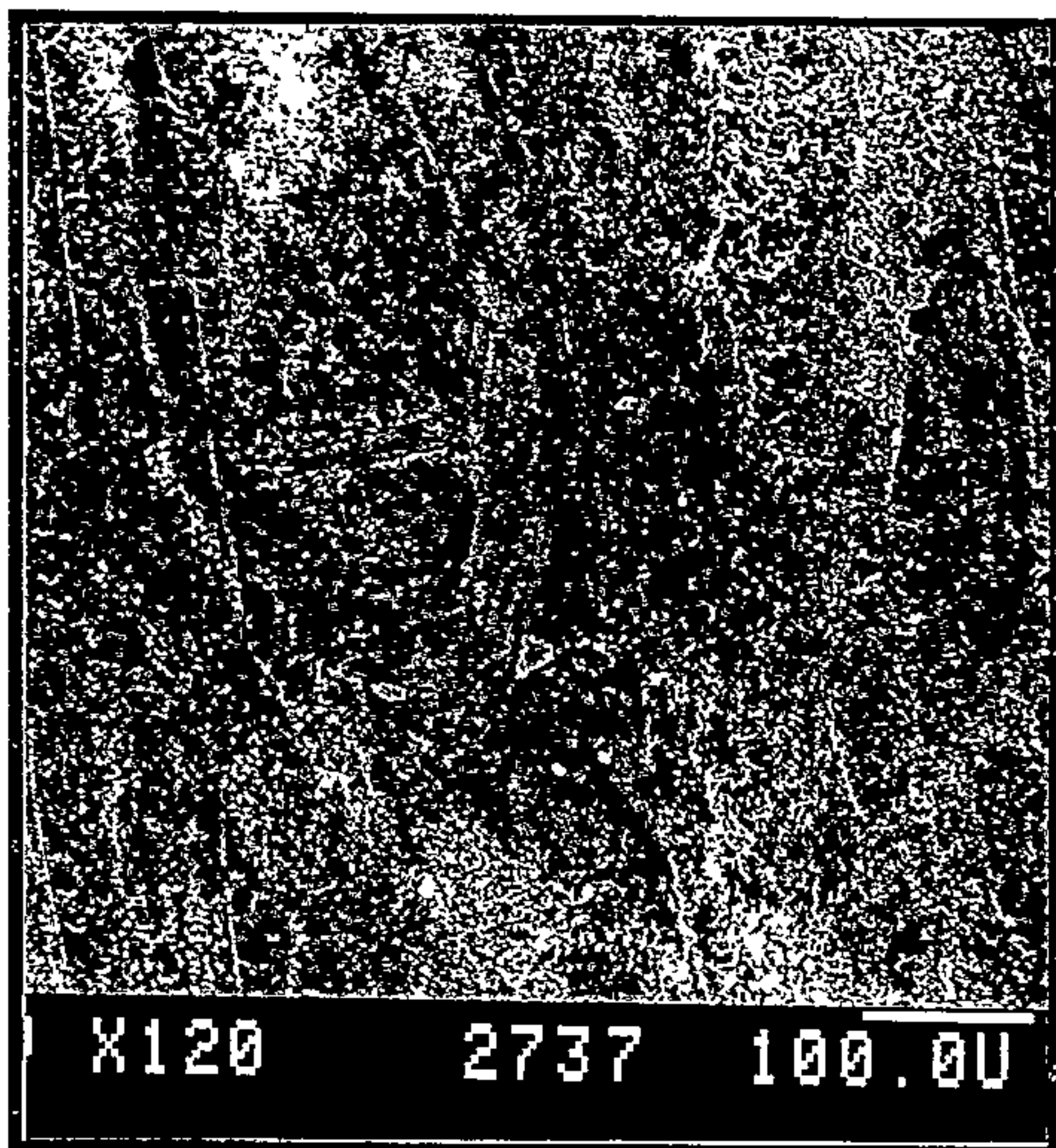


Fig: 5.14 Group I Middle Third (x120)

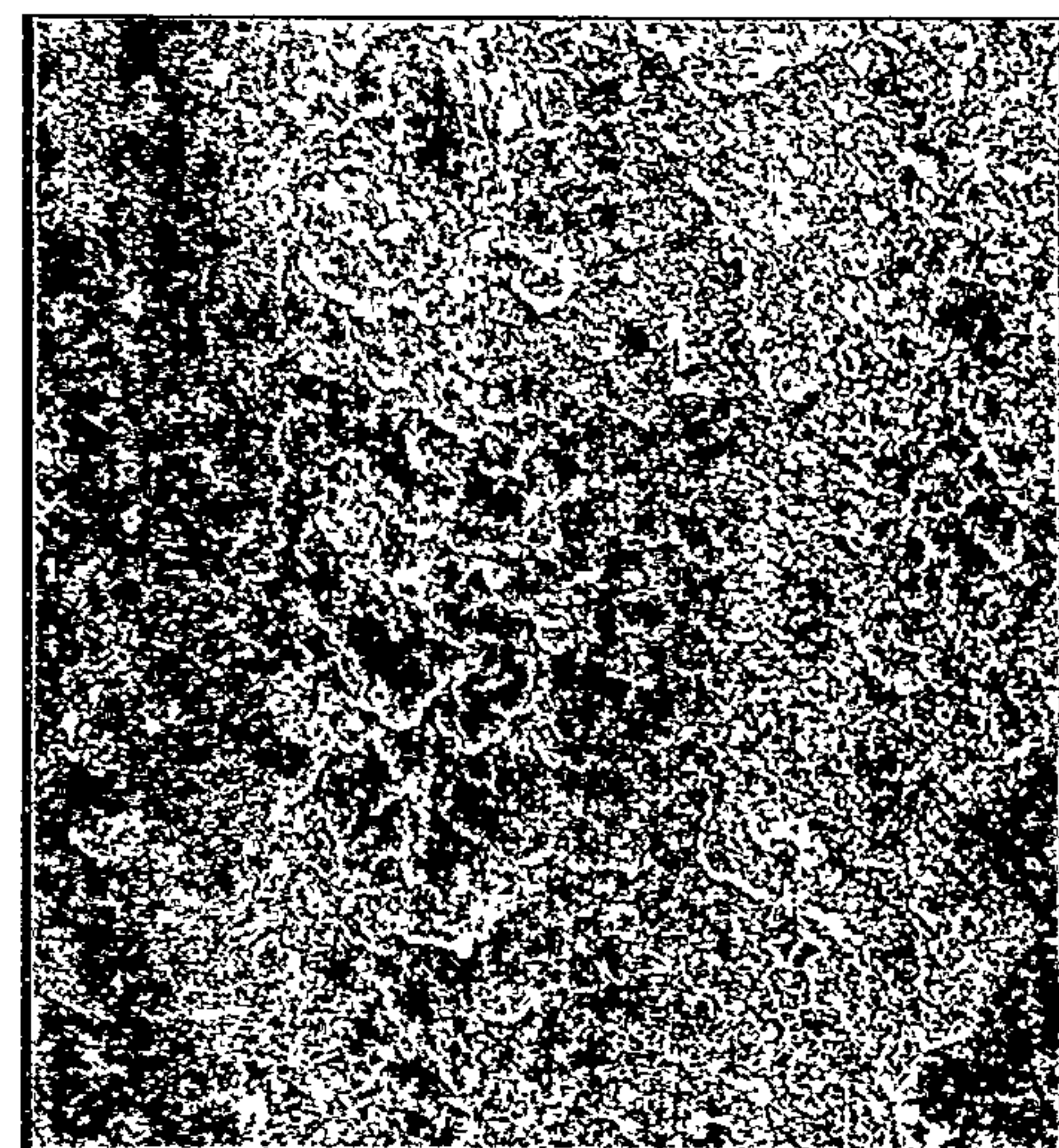


Fig: 5.15 Group I Middle Third (x300)



Fig: 5.16 Group I Apical Third (x120)

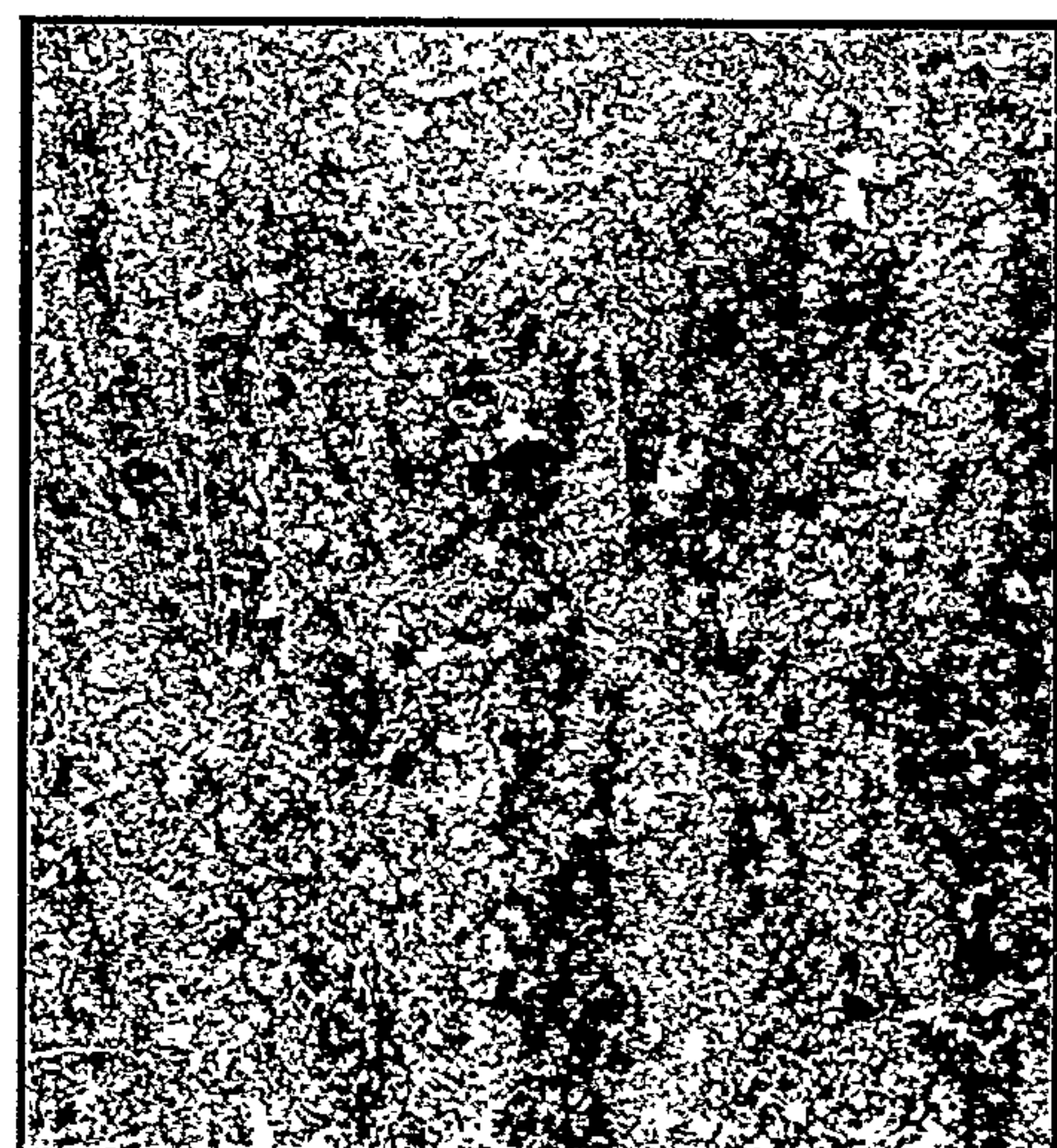


Fig: 5.17 Group I Apical Third (x300)

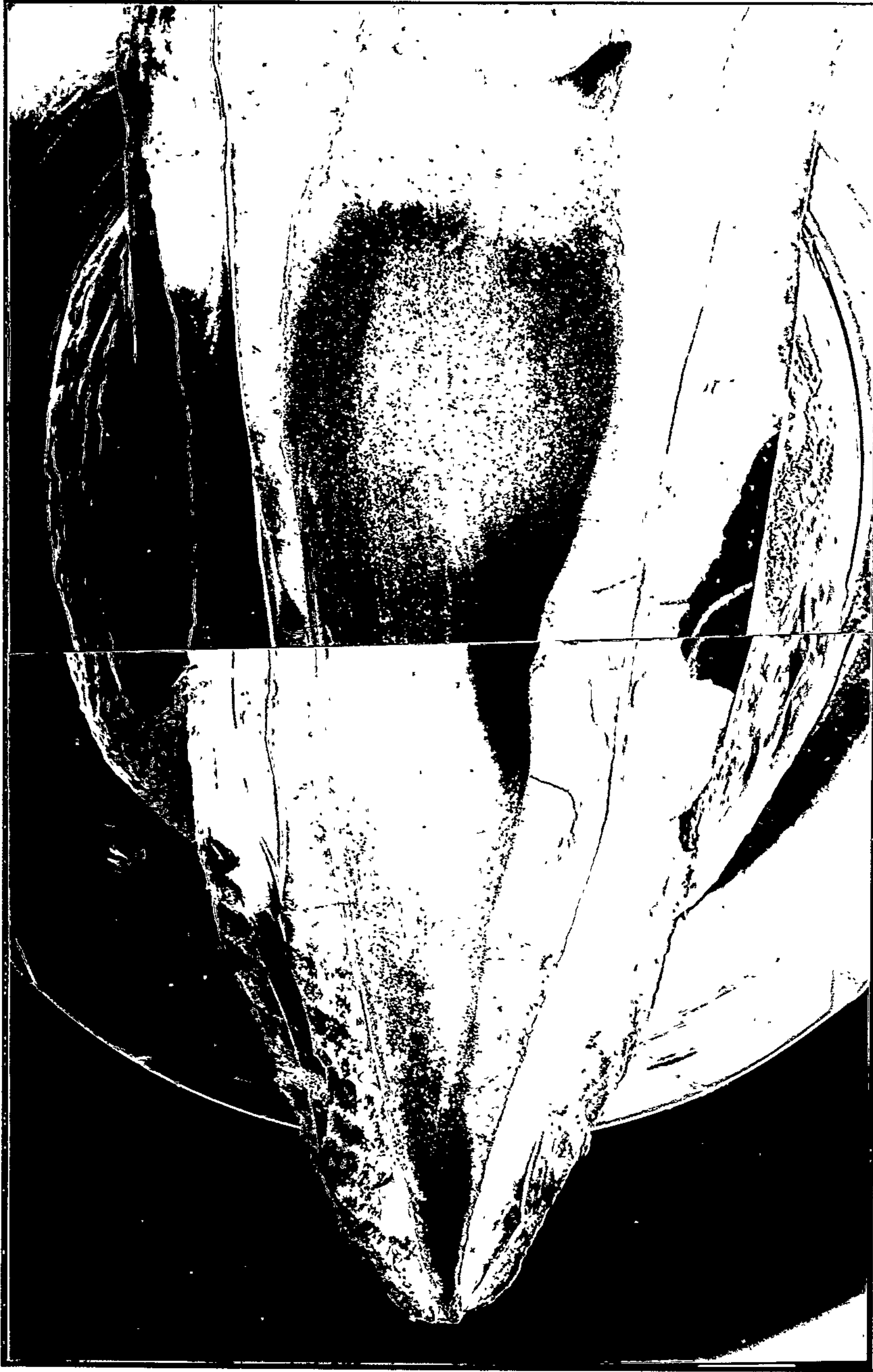


Fig: 5.18 - Group II Smear Layer Removed (x10 original magnification).

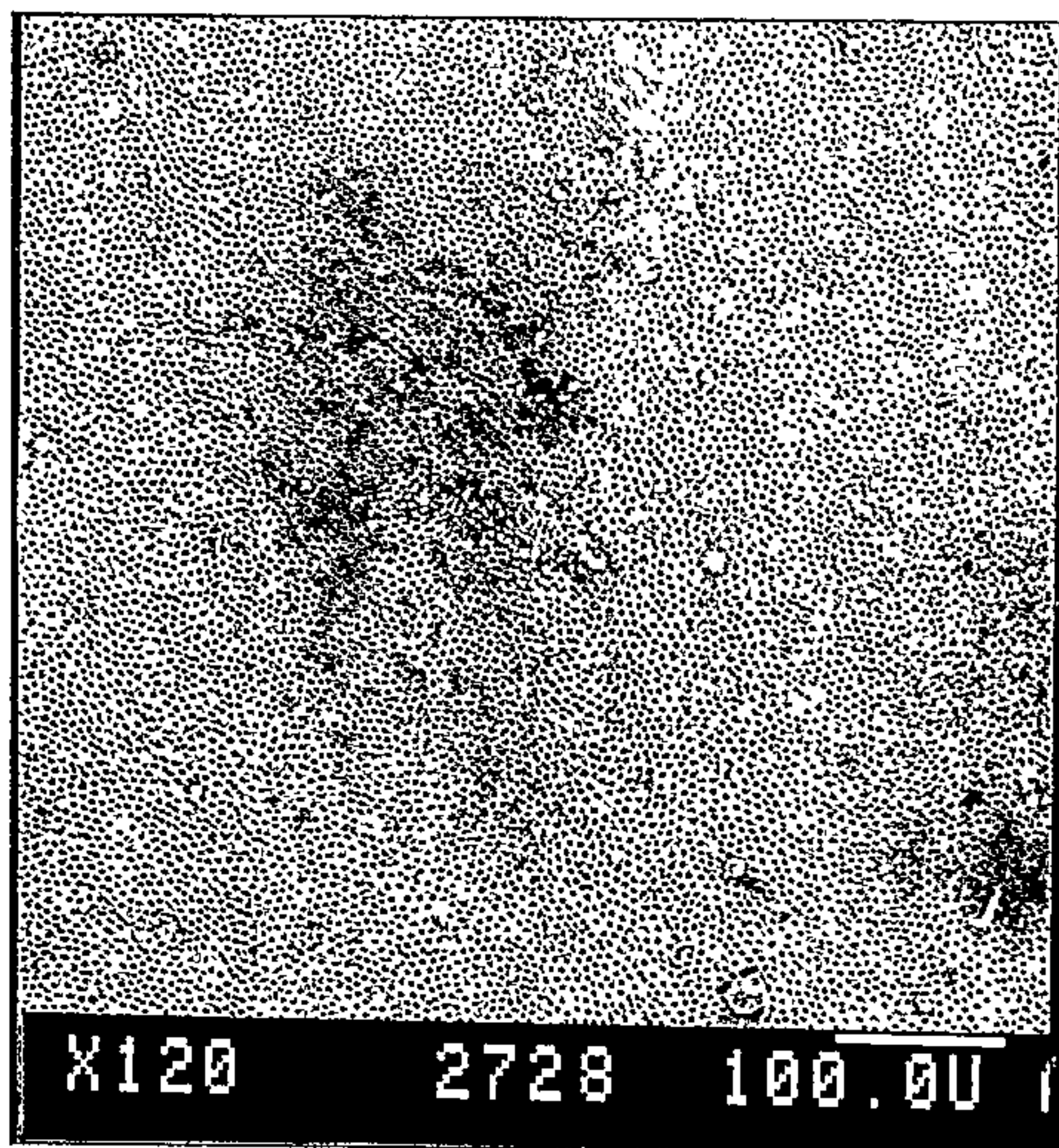


Fig: 5.19 Group II Coronal Third (x120 Orig)

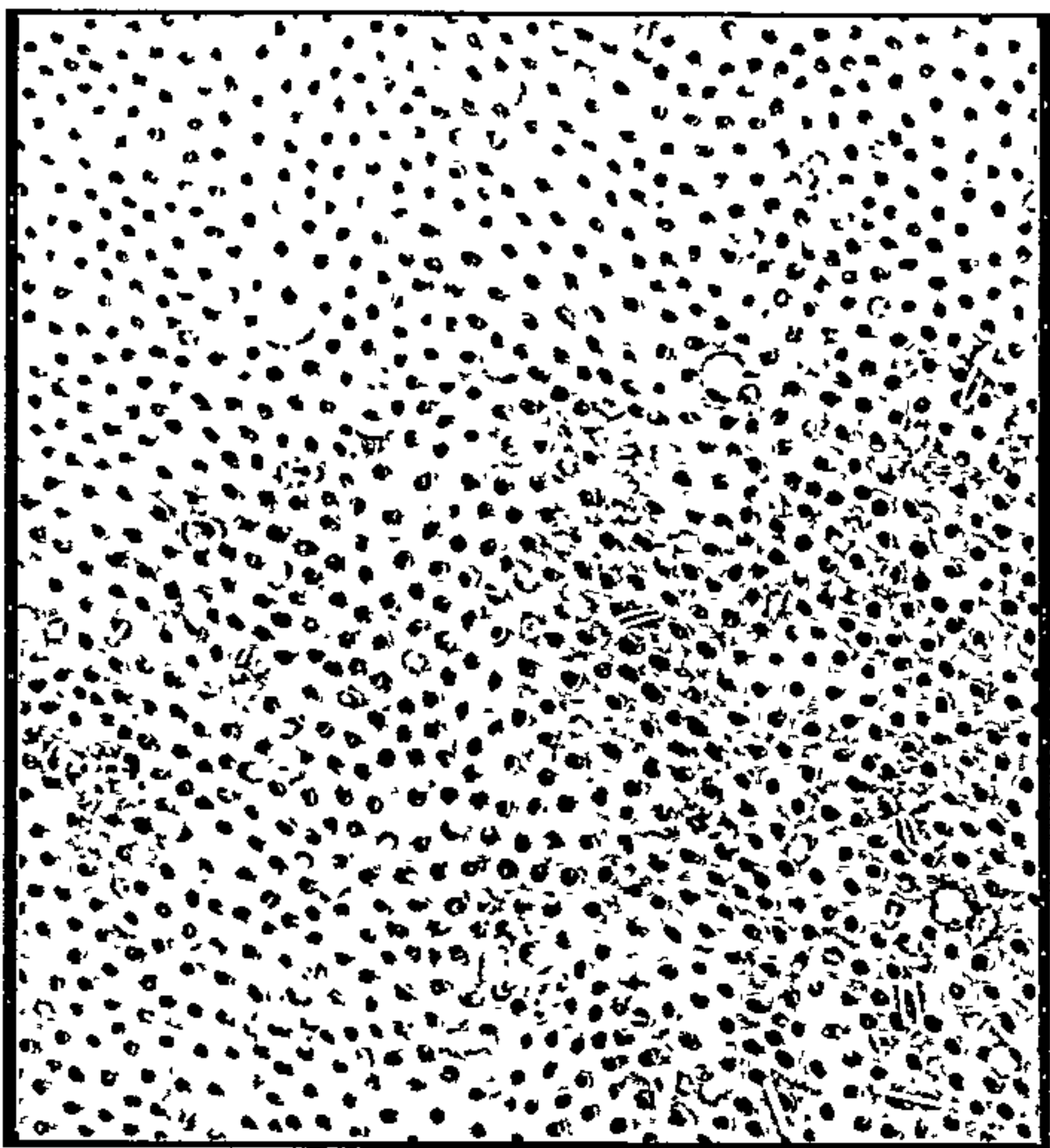


Fig: 5.20 Group II Coronal Third (x300 Orig)

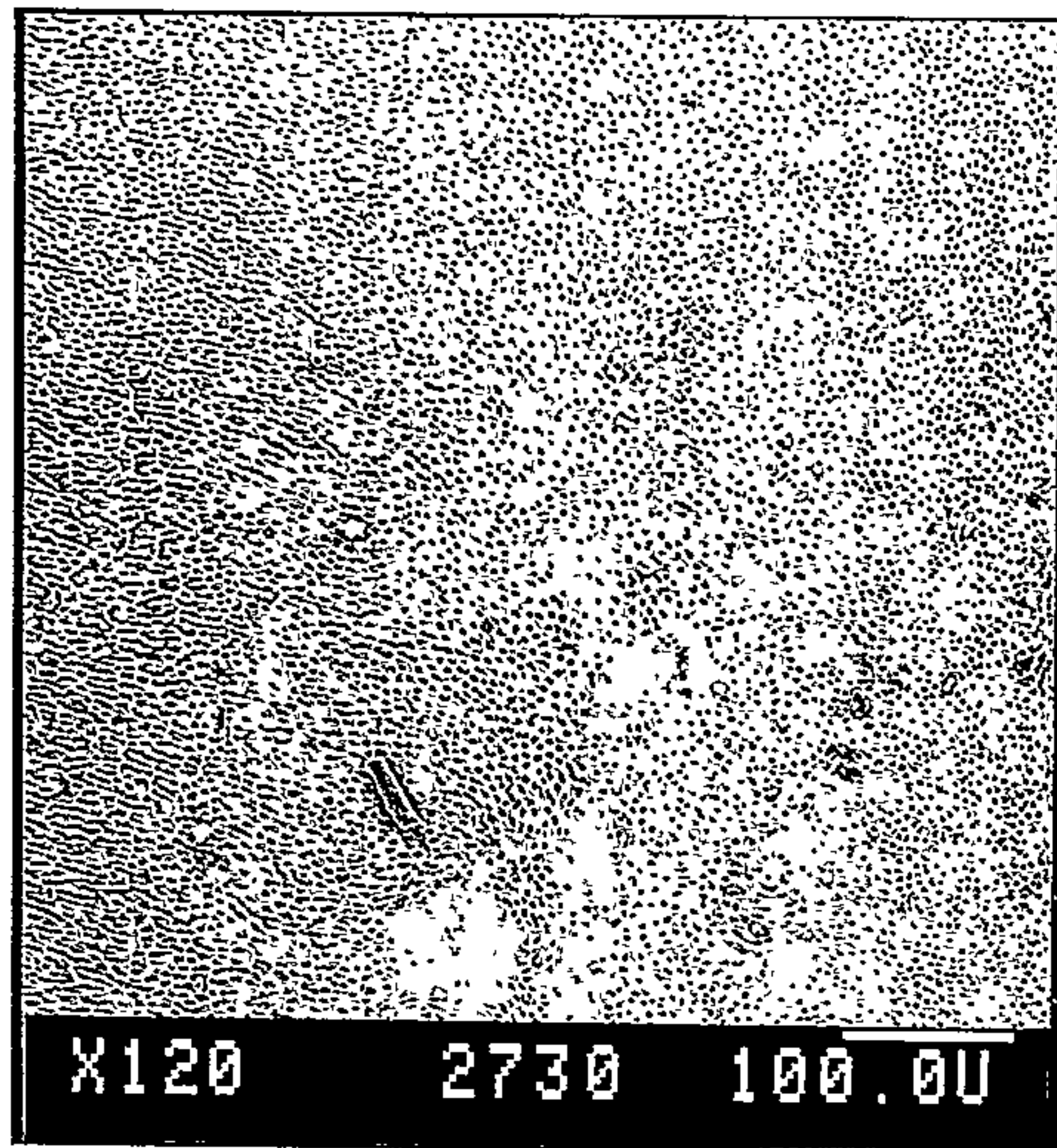


Fig: 5.21 Group II Middle Third (x120)

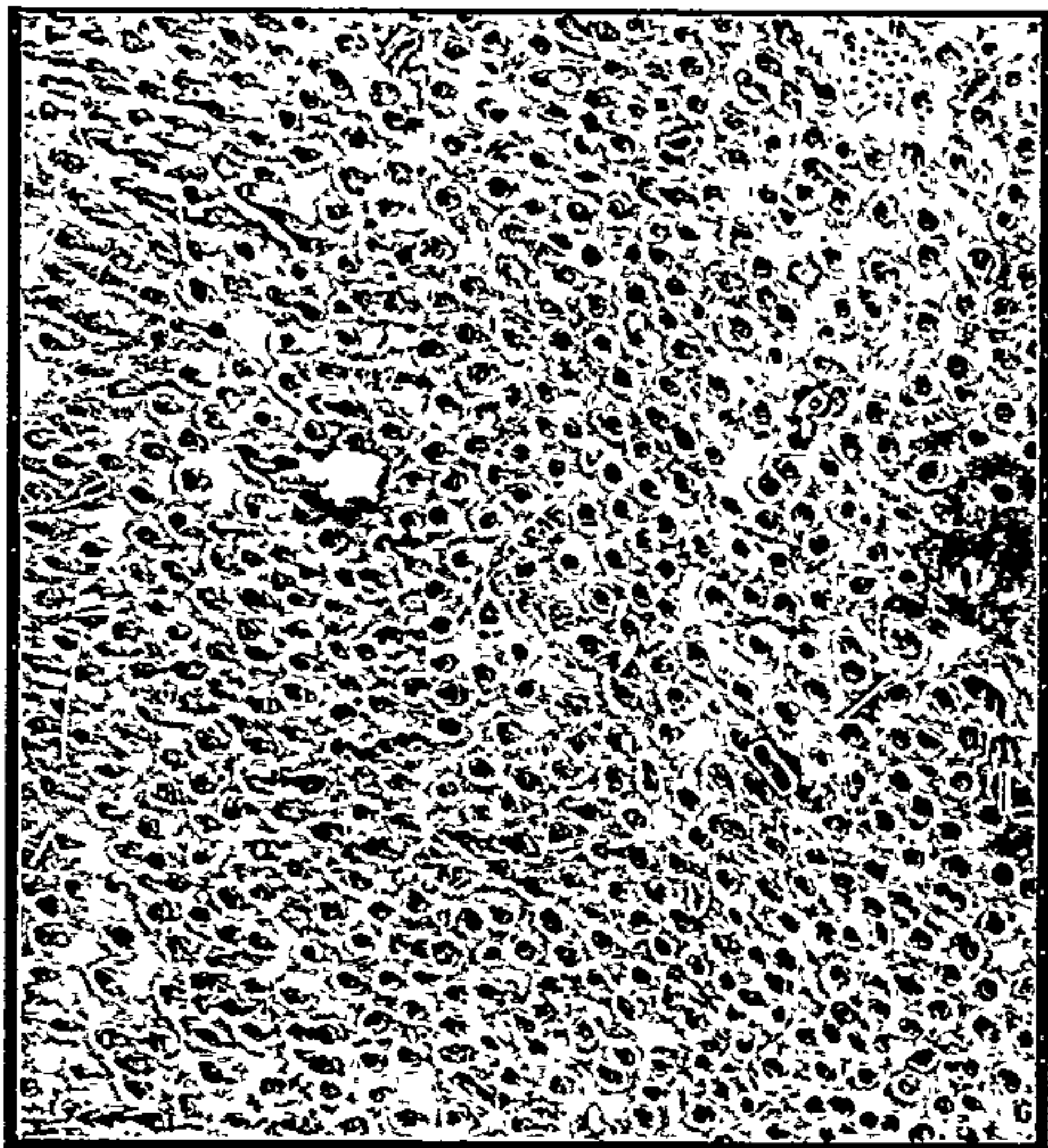


Fig: 5.22 Group II Middle Third (x300)



Fig: 5.23 Group II Apical Third (x120)

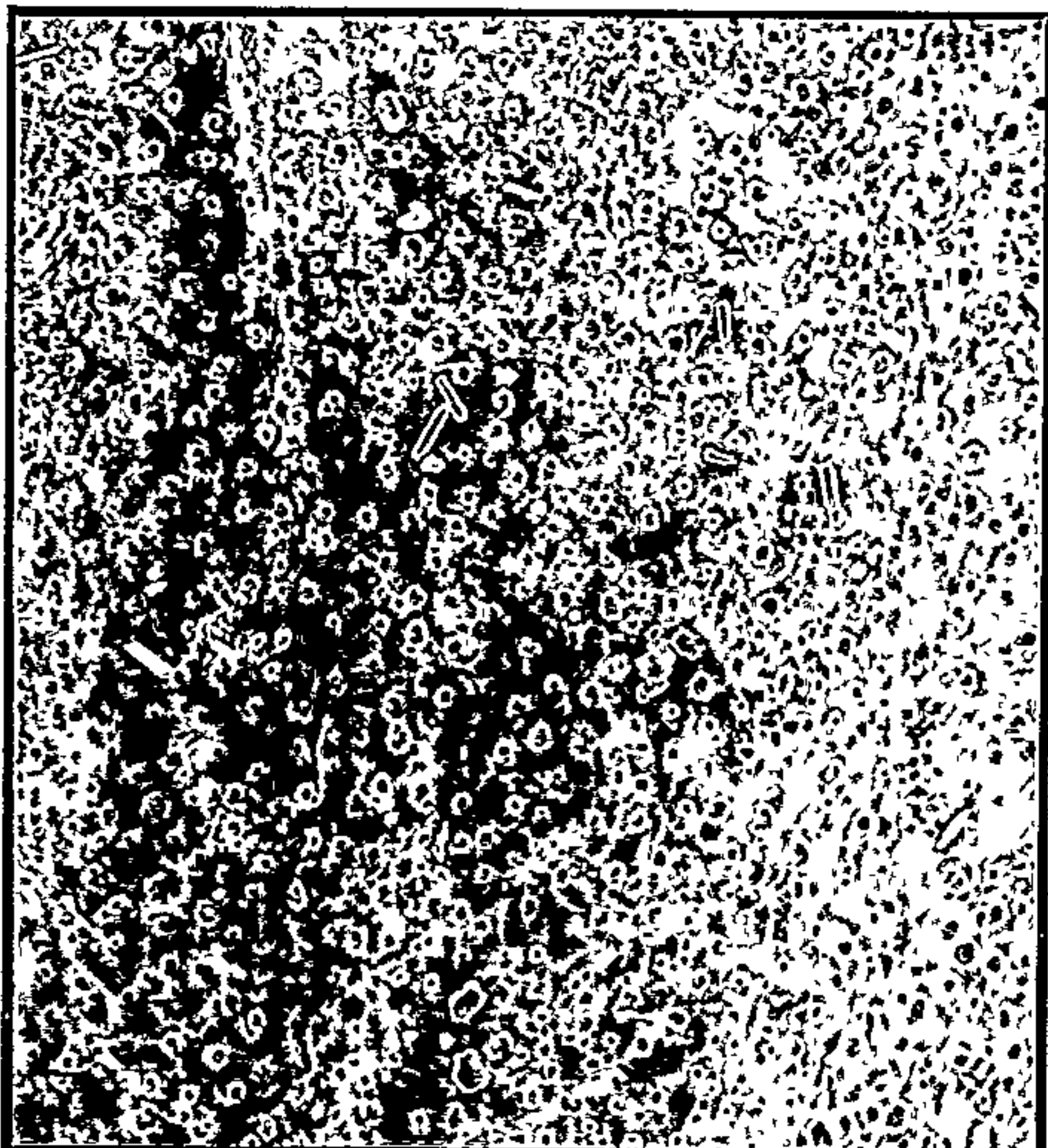


Fig: 5.24 Group II Apical Third (x300)

CHAPTER 6

DISCUSSION

The in vitro intact tooth model used in this investigation was designed to measure the availability of eugenol at the periodontal root surface. This was assessed following its release from a ZOE based root canal sealer, Procosol, under circumstances which were closely analogous to the clinical situation during endodontic treatment. The intended independent variable in this study was the influence of the smear layer upon eugenol release and, hence, the eugenol concentration(s) developed across root dentine.

The SEM photomicrograph data unequivocally demonstrated the complete removal of smear layer from the entire root canal of the experimental (Group II) specimens, utilizing the technique proposed by Yamada et al (1983). Conversely, the SEM data confirmed a uniform smear layer within the control (Group I) specimens.

The SEM data from Group II (smear removed) specimens also showed a decrease in the frequency or density of dentine tubules, at the dentino-pulpal junction, as the apical third of the root was approached (vid; Figs 5.18 through 5.24). Similarly a progressive decrease was observed in the average tubule diameter as the apex was approached. The present observations, whilst subjective, were consistent with existing quantitative data.

Several studies have shown that the number of dentinal tubules decrease from the pulp to the periodontal surface (Tronstad, 1973; Furseth, 1974; Garberoglio & Brännström, 1976; Whittaker & Kneale, 1979; Carrigan et al, 1984). Whittaker & Kneale (1979) and Carrigan et al (1984) also reported a 10% reduction in the tubule density between coronal and cervical root dentine, and a further 85% reduction between cervical and apical root dentine.

Garberoglio & Brännström (1976) examined the changes in tubular dimensions at various distances from the dentino-pulpal surface. They found the mean tubule diameter to be 2.5 μm at the dentino-pulpal junction whilst the mean peripheral tubule diameter was only 0.9 μm . Whittaker & Kneale (1979) also stated that the tubule diameter decreased as the apex was approached but did not specify the dimensions. Recently, Fogel et al (1988) also presented SEM morphometric data to support several conclusions regarding the hydraulic conductance (filtration) of cervical root dentine. They observed a decrease in mean tubule density ranging from 42,000 tubules. mm^{-2} , in cervical root dentine, down to 8,000 tubules. mm^{-2} , in apical root dentine. Fogel also reported a centrifugal decrease in mean tubule density, within cervical root dentine, ranging from 40,000 tubules. mm^{-2} in circum-pulpal dentine down to 20,000 tubules. mm^{-2} in peripheral dentine. This 'transverse' reduction in tubule number was also paralleled by a decrease in tubule diameter such that the mean circum-pulpal tubule diameter was 1.56 μm whilst the mean peripheral tubule diameter was only 1.07 μm .

These data substantiate the present qualitative observations that significant anatomical differences exist between coronal and radicular dentine. Furthermore, these morphometric differences may be significant in explaining the observed differences in the present quantitative data with respect to eugenol diffusion through root dentine.

The quantity and concentrations of eugenol released into the pbs solution were assayed by means of beta (β^-) scintillation spectrometry. The basic process involved in LSC is one in which the low energy particle emissions derived from weak β^- radio-isotopes (e.g. tritium) are converted into photons which can then be detected efficiently within a photomultiplier amplification system.

It was considered necessary to extract the non-dissociated $^3\text{H-E}$ from the eluant samples using a technique of hydrophobic solvent partitioning, since tritium labelling by catalytic exchange, used in the preparation of this stock tritiated eugenol solution, is stereo-chemically non-specific. Protons derived from eugenol are thought to take part in both the chelation and hydrolysis reactions with zinc eugenolate (von Fraunhofer, 1975). The present technique permitted the accurate detection of "eugenol only" molecules, therefore avoiding any inaccuracies that would be introduced by simultaneously determining any "non-eugenol" tritium atoms which had spontaneously transferred from a donor eugenol molecule to water. Previous work by Becker et al (1983) and Hume (1984a), had established that $^3\text{H-E}$ could be successfully extracted from an aqueous solution using a hydrophobic solvent partitioning technique based upon chloroform (CHCl_3).

During pilot work, considerable difficulty was initially experienced in measuring the very low levels of β^- emission encountered within the eluant samples from the early period of study. In order to maximize the detection efficiency, it proved necessary to use an alternative xylene based solvent extraction technique, rather than chloroform, and low ^{40}K -glass scintillation vials, in preference to polyethylene. These modifications significantly reduced the procedural errors related to quenching and background radiation.

Reference LSC data (Fox, 1976) indicated that chloroform was a relatively potent quenching agent particularly when counting low level tritium labelled compounds. Fox recommended that an aromatic solvent be used in preference to alkanes such as chloroform or carbon tetrachloride. Xylene (C_8H_{12}), an alkylbenzene related to toluene, was selected as a possible alternative solvent since :

1. Eugenol is soluble in xylene whilst only miscible in chloroform¹
2. Xylene is a more hydrophobic solvent than chloroform (0.02 vs 0.82 parts per 100 parts of water, respectively)²,
3. Xylene is a component of many scintillation cocktails and freely miscible with Aquasol³, the scintillant used in this study.

Pilot experiments with standard solutions of both ^3H -E and ^3H -water confirmed that xylene extraction was an efficient and highly selective barrier against ^3H -water (< 0.001% extracted) whilst insignificant amounts of ^3H -E were lost to the aqueous phase (< 0.01%) following duplicated extraction. Xylene exhibited approximately 2 to 5 times less quenching potential than chloroform, with respect to the standard ^3H -E samples, in Aquasol scintillant.

The selection of xylene, as the partitioning solvent for extraction of tritiated eugenol, was therefore considered appropriate.

1 Lange's Handbook of Chemistry.(1985) Dean: 13th ed ; 7-491

2 Lange's Handbook of Chemistry.(1985) Dean: 13th ed ; 7-214 & 7-688

3 Du Pont NEN Products (NEF-934)- Boston, MA, U.S.A.

In the present experiment, it was impossible to determine either the external or internal surface areas with sufficient accuracy to justify the precise calculation of a tritiated eugenol ($^3\text{H-E}$) "efflux". Therefore, tritiated eugenol movement into the pbs solution bathing the periodontal root surface, was quantified in terms of its gross "outflow" per tooth, i.e. amount per unit time, and the units described in picomoles [per tooth] per minute (pmol.min^{-1}).

The $^3\text{H-E}$ release through root dentine followed a similar pattern to previously reported data based upon coronal dentine (Hume, 1984a). However, there were several subtle but significant variations.

The peak $^3\text{H-E}$ outflow data for diffusion of eugenol through root dentine, from the present investigation, was approximately 11% that previously reported for coronal dentine. Hume (1984a) reported a range in the peak $^3\text{H-E}$ release rate, across coronal dentine, of 80 to $1,500 \text{ pmol.min}^{-1}$ (mean = 180, SD = 310); whereas the present study determined a range in peak $^3\text{H-E}$ outflow through root dentine of between 1 and 56 pmol.min^{-1} (pooled mean = 19.99, sem = 10.1).

The only in vivo eugenol release data were reported by Hashimoto et al (1988) who investigated $^3\text{H-E}$ concentrations in coronal pulp tissue of rats. Unfortunately, these data can not be directly correlated with data from the present investigation since Hashimoto et al examined ZOE material(s) placed into cavities with intentional pulp exposure. However, Hashimoto et al found that the peak $^3\text{H-E}$ concentration occurred in pulp tissue 30 minutes after placement, corresponding to 35 pmol.mg of pulp tissue or about $10 \mu\text{M}$ eugenol.

Fogel et al (1988) found that the hydraulic conductance (filtration) of circum-pulpal cervical root dentine was about 20% that of coronal dentine whilst peripheral root dentine was only 2% that of coronal dentine. The overall mean hydraulic conductance of cervical root dentine was 23% that previously reported in studies restricted to coronal dentine (Reeder et al, 1978; Pashley et al, 1981c,f; 1983c; 1984c).

The data of the present study were consistent with Fogel et al (1988). However, since filtration varies with the fourth power of tubule radius whilst diffusion varies with the square of the radius, the effect upon filtration data would be expected to be more pronounced than that for diffusion. The latter was not apparent in the present study but a likely explanation for this unpredicted result may again be related to the type of dentine studied. Fogel examined sections of cervical root dentine only, whereas the present data represent an overall mean of the entire root surface from the CEJ down to and including, most significantly, the apical root dentine.

In the current study, peak $^3\text{H-E}$ outflow consistently occurred much earlier than that reported by Hume (1984a), who found that little or no eugenol was released during the first hour after ZOE placement in coronal cavities. Conversely, Hashimoto et al (1988) detected $^3\text{H-E}$ in pulp tissue within 15 minutes of placement, peaking at 30 minutes and decreasing to basal levels within four hours. As stated earlier, the clinical relevance of the latter study was severely limited since the ZOE materials were placed into cavities with intentional pulp exposures. The mean peak $^3\text{H-E}$ outflow from roots occurred at $T_1 = 43$ minutes following canal obturation for both smear present (Group I) and smear removed (Group II) specimens.

The differences in these data could be explained by either an increased tritium LSC sensitivity used to measure the pbs samples during the initial periods of the present study or, the physical differences between the hydraulic forces created during the placement of ZOE 'restorative' materials into coronal cavities and a root canal which has been obturated by lateral condensation with a ZOE sealer (present study). It seems unlikely, however, that the initial chronological differences in outflow data can be related to specific qualitative differences in the nature of root versus coronal dentine, since later data and the overall release phenomena were similar in both studies.

The range in peak $^3\text{H-E}$ outflow occurred within the first 24 hours following canal obturation. This result was consistent with Hume's (1984a) findings, as was the observation that $^3\text{H-E}$ outflow, for each specimen, was maintained within one order of magnitude of its respective peak outflow for at least 10 days following obturation.

An interesting observation, in the present study, was the depression in mean $^3\text{H-E}$ outflow seen at $T_2 = 144$ mins and the subsequent second peak at $T_3 = 432$ mins. A similar depression did not occur in the early $^3\text{H-water}$ outflow data which suggests a eugenol-only related effect possibly independent of water movement itself.

It is reasonable to speculate that the first peak in mean $^3\text{H-E}$ outflow may represent an initial physical effect due to the hydraulic pressures of obturation (by lateral condensation) forcing unset sealer into contact with residual moisture in the underlying dentinal tubules. However the second peak in $^3\text{H-E}$ outflow, and its concurrence with the single mean peak outflow for $^3\text{H-water}$ at $T_3 = 432$ mins, suggest a centripetal movement of water, from the periodontal root surface, and its ultimate hydrolysis reaction with the zinc-eugenolate molecule in the set ZOE sealer.

The progressive increase in $^3\text{H-water}$ outflow, and its single moment of peak release, also imply that tritium ($^3\text{H}^+$) release kinetics were independent of either the obturation forces or $^3\text{H-E}$ outflow, *per se*, and may reflect a relatively stable water permeation due to filtration. Bulk fluid movements or filtration are not driven by a solute's concentration gradient but rather by the pressure differential, either hydraulic or osmotic. Therefore, none of the chemical energy or concentration gradient would be dissipated during a solvent's movement through dentine.

Another possible explanation for the observed depression in $^3\text{H-E}$ release, at $T_2 = 144$ minutes, relates to the possible coincidence between the reduction in $^3\text{H-E}$ outflow and completion of the root canal sealer's (Procosol) setting reaction. Hume (1984a) also reported a significant shift in eugenol outflow after 10 to 19 minutes which was attributed to the decrease in unreacted, labile eugenol due to the mixture's setting at about that time. However, direct correlation with Hume's data was inappropriate since these were related to 'restorative' mixtures of ZOE immersed directly in saline whereas the ZOE root canal sealer (Procosol) used in the present study, incorporated sodium borate to retard the usual ZOE setting reaction.

The setting time of Procosol has been reported to range from 70 minutes, at $37^\circ\text{C}/100\%$ humidity, (McComb & Smith, 1976) up to 40 hours at $22^\circ\text{C}/\text{ambient}$ humidity (Grossman, 1976).

Unfortunately, the present study was conducted at 22°C but 100% humidity, therefore, no relevant reference data were available for comparison which could directly correlate a coincidence between the observed depression in $^3\text{H-E}$ outflow and a 'confirmed' completion of sealer setting under the conditions of this study. Hence, the suggestion above must also be considered speculative.

The possibility that $^3\text{H-E}$ could diffuse through the apical foramen or be lost through the cervical/coronal seal, was controlled by covering the apex with three coats of nail varnish whilst the crown/Cavit seal were completely covered with sticky wax immediately following obturation.

This assumption was substantiated by the negative "control" specimens. The four negative control specimens (2 negative leakage, and 2 negative contamination) showed no signs of tritium release throughout the duration of study. Conveniently, these "control" samples also served as additional "blind" internal background standards during each batch or cycle of LSC.

One eluant sample did show contamination at $T = 0$, probably due to physical contact of the isotope with the individual vial. This contamination appeared to be an isolated event since no other samples from this specimen showed any sign(s) of persistent contamination. The exceptional contamination value was recorded but not included in the statistical analysis.

Clinically, the most relevant conclusion from the present data was that removal of the smear layer did not significantly influence the rate of $^3\text{H-E}$ outflow, at least within the intact root model used in the current investigation. This observation was contrary to that which would be predicted based upon the conceptual findings regarding permeation through slices of coronal dentine, in vitro (Merchant et al, 1977; Pashley & Livingston, 1978; Pashley et al, 1978b; Reeder et al, 1978; Boyer & Svare, 1981; Pashley et al, 1981c; 1983a; Dippel et al, 1984 and Pashley & Galloway, 1985). It is reasonable to propose that factor(s), as yet undetermined, may be clinically more significant to eugenol diffusion through an intact root than smear layer *per se*.

The conflicting results of the cited studies above and the present investigation may be reconciled through a combination of the anatomic or morphometric differences between coronal dentine and radicular dentine, and/or by the differences in experimental design. The above studies used techniques based upon the conceptual and idealized model of Outhwaite et al (1974), using uniform discs or slices of thin dentine cut from unerupted teeth, whereas the present study examined an anatomically complete structure, the intact root, which more closely replicates the in vivo situation.

The present data demonstrated that smear layer did not exert a rate-limiting step in terms of its restriction upon the hydrolysis reaction of water with zinc-eugenolate. As surface factors are thought to present the greatest barrier to solute permeation through dentine (Pashley et al, 1978a), the present data also suggest that peripheral or periodontal root dentine could be a clinically more significant determinant of hydrolytically released medicaments than a smear

layer created endodontically. This suggestion is indirectly supported by filtration data (Frydenlund & Krell, 1986; Fogel et al, 1988) that removal of cementum only did not significantly affect the L_p of cervical root dentine, in vitro, but removal of periodontal dentine did result in a significant L_p increase.

These data also imply that, *all other factors being equal*, removal of the smear layer from instrumented root canals should not potentiate nor exacerbate the existing therapeutic or cytotoxic effects of medicaments upon the periodontal tissues. This hypothesis implies that the relatively low permeability of intact radicular dentine, in vivo, may tend to protect the periodontium from both noxious and therapeutic agents introduced into the root canal during endodontic therapy. However, the toxicity of such products may also depend upon their pulpal concentration as diffusion is driven by the concentration gradient established across the membrane (Fick's law).

The specific concentrations of $^3\text{H-E}$, detected within root dentine, are of particular clinical relevance with regard to considerations of both anti-bacterial action and cytotoxicity within the root canal and the periodontal ligament tissues.

The in vitro eugenol concentrations in dentine determined in the present study were based upon several assumptions which may not be relevant or valid to in vivo extrapolation.

Hume (1984b) based his calculations of the eugenol concentration in coronal dentine upon a previously established approximation for the mean tubule volume (10% v/w) which represents the aqueous component of coronal dentine (Garberoglio & Brännström, 1976; Michelich et al, 1978; Pashley et al, 1978b). However, several authors have calculated wide regional variations in mean tubular volume, even within coronal dentine, which ranged from 1-4% at the DEJ up to 22-28% at the dentino-pulpal junction (Garberoglio & Brännström, 1976; Pashley et al, 1978b).

Anatomically, tubule frequency and diameter are known to progressively reduce in an apical direction. However, it is highly significant that the thickness of root dentine, in the present intact root model, also decreased as the apex is approached (Outhwaite et al, 1976; Pashley et al, 1978b). Whilst it seems logical that the mean tubular volume in root dentine should decrease as the apex was approached, it was entirely hypothetical to calculate a value for the mean tubular volume in root dentine as relevant data are limited.

Hume (1984a) calculated that a level of $10 \text{ nmol } (^3\text{H-E}).\text{mg}^{-1}$ of coronal dentine would be equivalent to an available eugenol concentration of 10^{-2} M within the intra-tubular spaces. The previously described morphometric differences between coronal and radicular dentine suggest this calculation should be modified in order to more accurately represent the available eugenol concentration within root dentine.

Based upon the cited anatomical differences and extrapolating from the calculations of Hume (1984a) and van der Graaf & Bosch (1988), the mean percentage tubule volume in radicular dentine was calculated to range from 5.4% in the cervical region down to 1.2% in the apical region. However, an arbitrary assumption was made that the tubule volume decreased in a linear manner as the apex was approached.

The "average" tubule volume of root dentine was therefore estimated at 3.3% or one third that of coronal dentine. Hence, it was calculated that $10 \text{ pmol } (^3\text{H-E}).\text{mg}^{-1}$ of powdered dentine represented a concentration of $3.3 \times 10^{-6} \text{ M}$ eugenol in root dentine.

In the present study, the mean $^3\text{H-E}$ concentration in outer or periodontal dentine, following peak outflow at $T_4 = 24$ hours, was found to be $1.6 \pm 0.23 \text{ pmol } (^3\text{H-E}).\text{mg}^{-1}$ or a eugenol concentration equivalent to $5.22 \times 10^{-6} \text{ M}$. Similarly, the mean $^3\text{H-E}$ concentration in mid-root dentine, following peak outflow, was found to be $3.71 \pm 0.64 \text{ pmol.mg}^{-1}$ or the equivalent of $1.21 \times 10^{-5} \text{ M}$ eugenol. The amount of eugenol within each standardized mix of root canal

sealer paste was analysed independently and was found to be equivalent to 0.23×10^{-3} moles per obturated canal.

The observed concentration of eugenol at the dentino-pulpal junction (0.23×10^{-3} M), after 24 hours, would be expected to kill most oral micro-organisms (Newman et al, 1979), a finding supported by clinical research (Ørstavik, 1981; Stevens & Grossman, 1981; Delevanis et al, 1983; Peters, 1986). The eugenol concentration found within mid-root and peripheral dentine (1.21×10^{-5} M ; 5.22×10^{-6} M) would not be great enough to sustain a definite antiseptic action deeper within the tubules nor at the periodontal root surface.

It also appears unlikely that the eugenol concentration, at the periodontal root surface (5.22×10^{-6} M), would cause any significant cytotoxic effects within the adjacent periodontal tissues (Valle et al, 1980; Hume et al, 1982; Hume, 1984b; Meryon, 1987). On the contrary, these concentrations may be advantageous or therapeutic . Eugenol concentrations of about 10^{-6} M are known to cause vasodilation (Hume, 1983), and inhibit the biosynthesis of cyclo-oxygenase products (Dewhirst, 1980; Hirafuji, 1984; Seow & Thong, 1986; Hashimoto et al, 1988). The local increase in vascular clearance and anti-prostaglandin action should further reduce the potential for any cytotoxic effects and may possibly enhance the local healing response.

Several authors have suggested that eugenol concentrations above 10^{-6} M were sufficient to compromise normal PMN membrane function, inhibit myeloperoxidase activity and interfere with the ability of these cells to migrate toward and interact with 'foreign' agents (Suzuki et al, 1985a,b; Seow & Thong, 1986; Fotos et al, 1987). The clinical implications of this eugenol-induced PMN depression are unknown.

It is not possible to speculate upon the potential for the eugenol concentrations observed within the current study to induce allergic or mutagenic responses within the adjacent periodontal tissues.

The clinical relevance of the present eugenol concentration data was limited in two respects. Firstly, dentine sampling was performed as an isolated and exclusive event following peak outflow ($T = 24$ hours) since the destructive methodology prevented any further serial determination of specimens. Secondly, the above data represent an accumulated amount of $^3\text{H-E}$ within dentine after 24 hours of passive elution from the external root surface into a limited volume reservoir of saline. This technique does not allow for any dynamic variables which may occur with vascular clearance or physiological changes within the periodontal tissues (Pashley, 1979; Hume, 1983).

Pashley (1988) claimed that radicular dentine was about 10^{-1} to 10^{-2} times less permeable than coronal dentine, when measured by filtration, but did not attempt to substantiate this claim. The results of the present study were, however, consistent with this conclusion and recent data derived from filtration study (Fogel et al, 1988).

The results of both the first and second parts of this investigation showed that there were no significant differences in either the eugenol outflow or the eugenol concentration gradient through root dentine which could be directly attributed to the removal of the smear layer from these endodontically treated teeth. This finding was contrary to the anticipated or predicted result based upon coronal dentine data.

The selection of eugenol as a tracer molecule was based upon its endodontic relevance but was perhaps an unfortunate choice due to its dependence upon hydrolysis and therefore upon water inflow for its release. It was entirely unexpected that factors other than smear layer would present a more significant rate-limiting influence upon eugenol outflow. However, the present data emphasize the potential importance of outer or periodontal dentine upon the local pharmacodynamics of intra-canal drugs, such as eugenol, which are released through a hydrolytic action.

Pashley et al (1978a) introduced the concept of a series of resistances to permeability across dentine and noted that the most significant determinant(s) of diffusion through coronal dentine were surface factors (e.g. smear layer). The conceptual dentine disc model used by Pashley was not directly analogous to the clinical situation within intact teeth but was intentionally idealized in order to simplify calculations and standardize test conditions. It is still possible that, under similar idealized conditions, removal of the smear layer from discs of root dentine would result in a significant increase in their permeability. However, the present data suggest that this premise should not be directly extrapolated to the clinical situation as it applies to the intact roots of endodontically treated teeth, in vivo. The results of the current intact root model indicate that the major determinant or barrier to the release of eugenol from ZOE based root canal sealers was not the smear layer *per se* but suggests that factor(s), as yet undefined, were likely to be more significant.

The clinical implications of these conclusions are such that removal of the smear layer from endodontically instrumented teeth, in vivo, appears unlikely to potentiate or increase either the therapeutic or cytotoxic effects of a eugenol based root canal sealer. Furthermore, it is also hypothesized that removal of the smear layer, following endodontic instrumentation, is unlikely to adversely affect a tooth's prognosis compared to otherwise conventional endodontic therapy without smear layer removal.

The clinical importance of leaving the smear layer in situ, within endodontically instrumented teeth, was not addressed by the present investigation and remains a relevant area for future investigation. It is also evident that much more work is required in order to evaluate and characterize the permeability properties of root dentine before the endodontic and periodontal implications of this very important tissue are realized.

CHAPTER 7

SUMMARY AND CONCLUSIONS

This study was designed to examine the influence of smear layer upon the diffusion of eugenol through intact root dentine, in vitro, within a model analogous to the clinical endodontic situation.

Smear layer was removed using the technique of Yamada et al (1983) involving sequential irrigation with 10 mls of a 15% EDTAC solution followed by 10 mls of a 1% NaOCl (Miltos). Scanning electron microscopy confirmed the successful removal of smear layer using this experimental irrigation technique.

Liquid scintillation spectrometry was used to measure the rate of outflow and the dentine concentration of labelled eugenol released from the tritium (^3H) labelled root canal sealer following endodontic instrumentation and obturation of human premolar teeth.

The results of the present study indicate that smear layer, *per se*, did not significantly influence either the release kinetics or dentine concentration of eugenol within the parameters of this intact root dentine model, in vitro.

Peak eugenol outflow ($19.99 \text{ pmol.min}^{-1}$) occurred within 24 hours of obturation and was sustained, within one order of magnitude, for at least ten days post-obturation. The eugenol concentration in dentine was found to range from a maximum of $0.23 \times 10^{-3} \text{ M}$, within the root canal, down to $5.22 \times 10^{-6} \text{ M}$ within the peripheral root dentine one day after obturation. These concentrations suggest the strong possibility of a local anti-microbial action within the root canal itself, at one day post-obturation, and also imply that ZOE based sealers should not be cytotoxic to the adjacent periodontal tissues.

The results of the present study indicate that removal of the smear layer from endodontically instrumented teeth, in vivo, was unlikely

to potentiate the therapeutic and/or cytotoxic effects of eugenol released from a ZOE based root canal sealer.

Therefore, it was hypothesized that smear layer removal, following endodontic instrumentation, was unlikely to detrimentally affect a tooth's prognosis when obturated with a ZOE root sealer compared to conventional endodontic therapy without smear layer removal.

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Appendix 1.1 SPECIMEN CASE HISTORY DATA

Case No.	Tooth (FDI)	Age	Sex	Fluoride	Extracted
115	45	2	1	1	26/3/87
116	35	2	1	1	2/4/87
101	15	2	0	1	11/3/87
106	25	2	0	1	25/3/87
102	45	2	0	1	11/3/87
107	35	2	0	1	25/3/87
172	44	3	1	1	17/3/87
179	34	3	1	1	24/3/87
152	44	2	1	1	21/4/87
203	34	2	1	1	29/4/87
148	34	3	0	1	14/5/87
156	44	3	0	1	21/5/87
181	45	1	1	1	16/5/87
133	35	1	1	1	10/5/87
244	35	2	0	1	6/8/87
235	45	2	0	1	30/7/87
243	45	2	1	1	4/8/87
249	35	2	1	1	10/4/87
241	45	2	1	1	4/8/87
253	35	2	1	1	11/8/87
186	34	2	1	1	31/7/87
185	44	2	1	1	27/7/87
263	35	2	0	1	20/8/87
270	45	2	0	1	20/8/87

Age : 1 = 12 to 14 years; 2 = 14 to 16 years; 3 = 16 to 18 years

Sex : 0 = Male; 1 = Female

Fluoride : 1 = Fluoridated water supply since at least 7 years of age

Appendix 1.2 SPECIMEN INSTRUMENTATION DATA

Specimen	WL	Volume	Irrigant	MAF	Flare	Fill Date	Delay
115	19.0	25 mls	Miltions	45k	50-70k	16/7/87	112
116	22.0	25	EDTA	45k	50-70k	23/7/87	119
106	18.5	20	M	30k	35-70k	16/7/87	121
101	19.5	20	E	30k	35-70k	23/7/87	134
107	21.5	20	M	45k	50-70k	30/7/87	135
102	20.5	20	E	45k	50-70k	30/7/87	141
172	20.0	25	E	35k	40-70k	3/8/87	139
179	20.0	25	M	35k	40-70k	3/8/87	132
152	19.0	20	M	35k	40-70k	4/8/87	108
203	19.0	20	E	35k	40-70k	4/8/87	101
156	21.0	15	E	35k	40-70k	9/8/87	114
148	21.0	15	M	35k	40-70k	9/8/87	121
133	21.0	20	E	45k	50-70k	11/8/87	123
181	21.0	20	M	45k	50-70k	11/8/87	117
244	23.0	15	M	45k	50-70k	25/8/87	19
235	23.0	15	E	45k	50-70k	25/8/87	26
243	20.0	15	M	45k	50-70k	26/8/87	22
249	20.0	15	E	45k	50-70k	26/8/87	16
241	22.5	20	M	45k	50-70k	26/8/87	22
253	22.5	20	E	45k	50-70k	26/8/87	15

Appendix 1.3 CANAL DIMENSIONS AND LATERAL SURFACE AREA

Specimen Length Apical Radius Major Axis Minor Axis Internal Surface Area

115	14.11 mm	0.225 mm	1.59 mm	0.74 mm	65.159 mm ²
116	13.00	0.225	1.73	0.63	62.751
101	11.64	0.150	1.85	0.67	56.979
106	10.29	0.150	2.09	0.46	54.583
107	13.86	0.225	1.78	0.74	69.583
102	14.60	0.225	1.59	0.73	67.316
179	11.35	0.175	1.23	0.53	40.132
172	10.31	0.175	1.27	0.67	38.815
152	14.07	0.175	1.14	0.85	52.193
203	14.49	0.175	1.33	0.83	58.434
148	12.36	0.175	1.32	0.71	48.248
156	14.25	0.175	1.32	0.74	55.778
181	12.27	0.225	1.53	0.82	56.427
133	11.15	0.225	1.60	0.76	52.094
244	14.90	0.225	1.61	0.34	65.113
235	13.31	0.225	1.60	0.28	57.715
243	13.00	0.225	1.32	1.20	60.922
249	12.09	0.225	1.42	1.19	58.502
241	14.66	0.225	1.73	0.72	71.672
253	15.00	0.225	1.83	0.86	78.270

Appendix 2.1 DATA FOR TRITIATED EUGENOL

Table 2.1a. Tritiated (³H)-eugenol data arranged in Groups by Interval.

Group I - Miltons by interval					Group II - EDTAC by interval				
Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₀ =1 min									
115	0.00	0.00	0.00	0.00	116	0.00	0.00	0.00	0.00
106	0.00	0.00	0.00	0.00	101	0.00	0.00	0.00	0.00
107	0.00	0.00	0.00	0.00	102	0.00	0.00	0.00	0.00
179	0.00	0.00	0.00	0.00	172	0.00	0.00	0.00	0.00
152	0.00	0.00	0.00	0.00	203	0.00	0.00	0.00	0.00
148	0.00	0.00	0.00	0.00	156	0.00	0.00	0.00	0.00
181	0.00	0.00	0.00	0.00	133	0.00	0.00	0.00	0.00
244	0.00	0.00	0.00	0.00	235	0.00	0.00	0.00	0.00
243	0.00	0.00	0.00	0.00	249	0.00	0.00	0.00	0.00
241	0.00	0.00	0.00	0.00	253	0.00	0.00	0.00	0.00
mean	0.00	0.00	0.00		mean	0.00	0.00	0.00	
SD ±	0.00	0.00	0.00		SD ±	0.00	0.00	0.00	
SEM ±	0.00	0.00	0.00		SEM ±	0.00	0.00	0.00	
Σ(rate)=	0.00				Σ(rate)=	0.00			
Σ(rate ²)=	0.00				Σ(rate ²)=	0.00			
(Σrate) ² /10=	0.00				(Σrate) ² /10=	0.00			
t-score=	0.0000								

DPM - Disintegrations per minute of tritiated (³H)-eugenol.

RATE - Measured in 10⁻¹² Moles of tritiated (³H)-eugenol min⁻¹

MEAN - Mean rate for each Group by Interval

SD - Standard deviation for each Group by Interval

SEM - Standard error of the mean for each Group by Interval

Rate², Σ(rate), Σ(rate²), (Σrate²) - data for statistical analysis.

Paired, two-tailed Student t-Tests were applied to mean ³H-E rate data for Group I and Group II, at each time interval.

The parameters for significance were selected as follows :

An α level = 0.05 ; Degrees of Freedom (df) = 18 ; t_α = 2.101

{H₀ : μ₁ ≠ μ₂} ; {H₁ : μ₁ = μ₂}

if calculated t-score < 2.101, then accept the null hypothesis

i.e. : "on the basis of the present study a difference could not be statistically guaranteed "

TABLE 2.1b. Tritiated (³H)-eugenol data arranged in Groups by Interval.

Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₁ =0.03 days									
115	3.60	3.60	0.09	0.01	116	4.36	4.36	0.10	0.01
106	12.49	12.49	0.30	0.09	101	23.43	23.43	0.56	0.31
107	7.60	7.60	0.18	0.03	102	6.85	6.85	0.16	0.03
179	0.00	0.00	0.00	0.00	172	2.41	2.41	0.06	0.00
152	16.39	16.39	0.39	0.15	203	8.95	8.95	0.21	0.05
148	6.46	6.46	0.15	0.02	156	12.01	12.01	0.29	0.08
181	0.00	0.00	0.00	0.00	133	101.98	101.98	2.43	5.90
244	1.77	1.77	0.04	0.00	235	0.00	0.00	0.00	0.00
243	15.18	15.18	0.36	0.13	249	40.07	40.07	0.95	0.91
241	71.98	71.98	1.71	2.94	253	0.00	0.00	0.00	0.00
mean	13.17	13.17	0.31		mean	20.01	20.01	0.48	
SD ±	21.67	21.67	0.52		SD ±	31.36	31.35	0.75	
SEM ±	6.85	6.85	0.16		SEM ±	9.92	9.92	0.24	
Σ(rate)= 3.13					Σ(rate)= 4.76				
Σ(rate ²)= 3.38					Σ(rate ²)= 7.28				
(Σrate) ² /10= 0.98					(Σrate) ² /10= 2.27				
t-score= -0.5674									
Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₂ =0.1 days									
115	2.53	6.13	0.03	0.00	116	2.06	6.42	0.02	0.00
106	7.33	19.82	0.07	0.01	101	26.15	49.58	0.26	0.07
107	22.77	30.37	0.23	0.05	102	41.90	48.75	0.42	0.18
179	15.42	14.34	0.15	0.02	172	1.02	3.43	0.01	0.00
152	17.11	33.50	0.17	0.03	203	15.36	24.30	0.15	0.02
148	24.93	31.39	0.25	0.06	156	12.01	12.01	0.29	0.08
181	0.00	0.00	0.00	0.00	133	0.00	101.97	0.00	0.00
244	11.40	13.17	0.11	0.01	235	0.10	0.10	0.00	0.00
243	10.62	25.80	0.11	0.01	249	55.86	95.92	0.56	0.31
241	51.51	123.48	0.52	0.27	253	2.77	2.77	0.03	0.00
mean	16.14	29.30	0.16		mean	15.72	34.52	0.17	
SD ±	15.01	35.26	0.15		SD ±	19.67	38.45	0.20	
SEM ±	4.75	11.15	0.05		SEM ±	6.22	12.16	0.06	
Σ(rate)= 1.61					Σ(rate)= 1.74				
Σ(rate ²)= 0.46					Σ(rate ²)= 0.66				
(Σrate) ² /10= 0.26					(Σrate) ² /10= 0.30				
t-score= -0.1568									

TABLE 2.1c. Tritiated (³H)-eugenol data arranged in Groups by Interval.

Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₃ =0.3 days									
115	15.80	21.93	0.06	0.00	116	7.47	13.89	0.03	0.00
106	11.15	30.98	0.05	0.00	101	30.68	80.27	0.13	0.02
107	233.75	264.12	0.96	0.92	102	124.33	173.08	0.51	0.26
179	111.02	125.35	0.45	0.21	172	34.12	37.55	0.14	0.02
152	80.04	113.54	0.33	0.11	203	49.95	74.26	0.20	0.04
148	43.87	75.26	0.18	0.03	156	49.88	77.99	0.20	0.04
181	22.96	18.02	0.09	0.01	133	22.61	124.58	0.09	0.01
244	44.10	57.27	0.18	0.03	235	29.85	29.95	0.12	0.01
243	15.77	41.57	0.06	0.00	249	130.72	226.64	0.54	0.29
241	114.50	237.98	0.47	0.22	253	27.88	30.65	0.11	0.01
mean	69.30	98.60	0.28		mean	50.75	86.88	0.21	
SD ±	69.50	88.32	0.28		SD ±	42.31	68.98	0.17	
SEM ±	21.98	27.93	0.09		SEM ±	13.38	21.81	0.05	
Σ(rate)= 2.84					Σ(rate)= 2.08				
Σ(rate ²)= 1.54					Σ(rate ²)= 0.70				
(Σrate) ² /10= 0.81					(Σrate) ² /10= 0.43				
t-score= 0.7208									
Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₄ =1 day									
115	27.15	49.08	0.03	0.00	116	14.85	28.74	0.02	0.00
106	15.49	46.47	0.02	0.00	101	63.89	144.15	0.08	0.01
107	774.95	1039.06	0.95	0.89	102	649.37	822.45	0.79	0.63
179	143.43	268.78	0.17	0.03	172	52.06	89.61	0.06	0.00
152	133.50	247.03	0.16	0.03	203	71.93	146.18	0.09	0.01
148	155.16	230.42	0.19	0.04	156	297.92	375.91	0.36	0.13
181	46.16	64.18	0.06	0.00	133	51.54	176.12	0.06	0.00
244	90.58	147.84	0.11	0.01	235	83.79	113.74	0.10	0.01
243	10.91	52.48	0.01	0.00	249	179.53	406.17	0.22	0.05
241	140.95	378.93	0.17	0.03	253	103.41	134.06	0.13	0.02
mean	153.83	252.43	0.19		mean	156.83	243.71	0.19	
SD ±	225.51	299.20	0.28		SD ±	191.27	236.10	0.23	
SEM ±	71.31	94.62	0.09		SEM ±	60.49	74.66	0.07	
Σ(rate)= 1.88					Σ(rate)= 1.91				
Σ(rate ²)= 1.03					Σ(rate ²)= 0.86				
(Σrate) ² /10= 0.35					(Σrate) ² /10= 0.37				
t-score= -0.0321									

TABLE 2.1d. Tritiated (³H)-eugenol data arranged in Groups by Interval.

Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₅ =3 days									
115	113.21	162.29	0.05	0.00	116	33.63	62.37	0.01	0.00
106	40.63	87.10	0.02	0.00	101	306.49	450.64	0.14	0.02
107	1608.33	2647.39	0.71	0.51	102	1073.20	1895.65	0.47	0.20
179	242.93	511.71	0.11	0.01	172	77.50	167.10	0.03	0.00
152	232.25	479.28	0.10	0.01	203	177.36	323.54	0.08	0.01
148	779.71	1010.13	0.35	0.12	156	590.47	966.37	0.26	0.07
181	56.59	120.78	0.03	0.00	133	74.95	251.06	0.03	0.00
244	299.03	446.87	0.13	0.02	235	225.70	339.44	0.10	0.01
243	85.38	137.85	0.04	0.00	249	449.50	855.67	0.20	0.04
241	327.50	706.43	0.14	0.02	253	325.35	459.41	0.14	0.02
mean	378.55	630.98	0.17		mean	333.41	577.12	0.15	
SD ±	482.61	767.78	0.21		SD ±	313.71	543.41	0.14	
SEM ±	152.61	242.79	0.07		SEM ±	99.21	171.84	0.04	
Σ(rate)=	1.68				Σ(rate)=	1.48			
Σ(rate ²)=	0.69				Σ(rate ²)=	0.39			
(Σrate) ² /10=	0.28				(Σrate) ² /10=	0.22			
t-score=	0.2480								
Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₆ =10 days									
115	297.11	459.40	0.04	0.00	116	170.54	232.91	0.02	0.00
106	131.39	218.48	0.02	0.00	101	813.43	1264.08	0.10	0.01
107	774.95	1039.06	0.95	0.89	102	1968.96	3864.61	0.25	0.06
179	402.19	913.90	0.05	0.00	172	113.99	281.10	0.01	0.00
152	552.76	1032.03	0.07	0.00	203	465.94	789.47	0.06	0.00
148	1252.69	2262.82	0.16	0.02	156	792.71	1759.08	0.10	0.01
181	955.82	1458.09	0.04	0.00	133	514.18	765.25	0.06	0.00
244	1014.15	1461.02	0.13	0.02	235	791.28	1130.72	0.10	
243	297.06	434.91	0.04	0.00	249	1150.42	2006.08	0.14	0.02
241	770.42	1476.85	0.10	0.01	253	990.71	1450.11	0.12	0.02
mean	644.85	1075.65	0.16		mean	777.21	1354.34	0.10	
SD ±	366.38	616.40	0.28		SD ±	535.50	1055.83	0.07	
SEM ±	115.86	194.92	0.09		SEM ±	169.34	333.88	0.02	
Σ(rate)=	1.58				Σ(rate)=	0.97			
Σ(rate ²)=	0.95				Σ(rate ²)=	0.13			
(Σrate) ² /10=	0.25				(Σrate) ² /10=	0.09			
t-score=	0.6663								

TABLE 2.1e. Tritiated (³H)-eugenol data arranged in Groups by Interval.

Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₇ =30 days									
115	1190.22	1649.62	0.05	0.00	116	574.21	807.12	0.03	0.00
106	719.64	938.12	0.03	0.00	101	721.91	1985.98	0.03	0.00
107	5113.43	10687.3	0.23	0.05	102	4429.70	8294.31	0.20	0.04
179	905.63	1819.52	0.04	0.00	172	168.07	449.17	0.01	0.00
152	749.69	1781.72	0.03	0.00	203	764.12	1553.59	0.03	0.00
148	2638.31	4901.13	0.12	0.01	156	1256.87	3015.95	0.06	0.00
181	955.82	1458.10	0.04	0.00	133	796.85	1562.10	0.04	0.00
244	2015.68	3476.70	0.09	0.01	235	1774.20	2904.90	0.08	0.01
243	658.39	1093.92	0.03	0.00	249	2216.20	4222.28	0.10	0.01
241	1614.91	3091.76	0.07	0.01	253	2117.20	3567.30	0.09	0.01
mean 1656.17 3089.72 0.07					mean 1481.93 2836.27 0.07				
SD ± 1374.91 2940.70 0.06					SD ± 1241.88 2262.99 0.06				
SEM ± 434.79 929.93 0.02					SEM ± 392.72 715.62 0.02				
Σ(rate)= 0.74					Σ(rate)= 0.66				
Σ(rate ²)= 0.09					Σ(rate ²)= 0.07				
(Σrate) ² /10= 0.05					(Σrate) ² /10= 0.04				
t-score= 0.2974									
Specimen	DPM	CUM.	RATE	Rate ²	Specimen	DPM	CUM.	RATE	Rate ²
T ₈ =100 days									
115	4796.96	6446.57	0.0599	0.004	116	650.72	1457.83	0.0081	0.000
106	1432.94	2371.06	0.0179	0.000	101	1292.41	3278.39	0.0162	0.000
107	5348.19	16035.49	0.0668	0.005	102	6004.59	14298.89	0.0751	0.006
179	2130.87	3951.48	0.0266	0.001	172	457.42	906.59	0.0057	0.000
152	1697.09	3478.81	0.0212	0.000	203	1566.90	3120.49	0.0196	0.000
148	4198.88	9100.01	0.0525	0.003	156	2488.13	5504.07	0.0311	0.001
181	1964.02	3422.11	0.0245	0.001	133	1567.93	3130.02	0.0196	0.000
244	3986.34	7463.03	0.0498	0.002	235	3740.82	6645.71	0.0467	0.002
243	1510.29	2603.58	0.0189	0.000	249	2742.94	6965.22	0.0343	0.001
241	2757.75	5849.51	0.0345	0.001	253	3034.94	6602.24	0.0379	0.001
mean 2982.33 6072.17 0.0373					mean 2354.68 5190.95 0.0294				
SD ± 1468.56 4143.73 0.0183					SD ± 1658.17 3870.01 0.0207				
SEM ± 464.40 1310.36 0.0058					SEM ± 524.36 1223.80 0.0066				
Σ(rate)= 0.3730					Σ(rate)= 0.2940				
Σ(rate ²)= 0.0170					Σ(rate ²)= 0.0130				
(Σrate) ² /10= 0.0139					(Σrate) ² /10= 0.0086				
t-score= 0.8944									

Appendix 2.2 DATA FOR TRITIATED WATER

TABLE 2.2a. Tritiated (³H)-water data arranged in Groups by Interval.

Group I - Miltons by interval				Group II - EDTAC by interval			
Specimen	DPM	RATE	Rate ²	Specimen	DPM	RATE	Rate ²
T ₀ =1 min							
115	0.00	0.00	0.00	116	0.00	0.00	0.00
106	0.00	0.00	0.00	101	0.00	0.00	0.00
107	0.00	0.00	0.00	102	0.00	0.00	0.00
179	0.00	0.00	0.00	172	0.00	0.00	0.00
152	0.00	0.00	0.00	203	0.00	0.00	0.00
148	0.00	0.00	0.00	156	0.00	0.00	0.00
181	0.00	0.00	0.00	133	143.74	143.74	20661.19
244	0.00	0.00	0.00	235	0.00	0.00	0.00
243	0.00	0.00	0.00	249	0.00	0.00	0.00
241	0.00	0.00	0.00	253	0.00	0.00	0.00
mean	0.00	0.00		mean	14.37	14.37	
SD ±	0.00	0.00		SD ±	45.45	45.45	
SEM±	0.00	0.00		SEM±	14.37	14.37	
Σ(rate)=		0.00		Σ(rate)=		143.74	
Σ(rate ²)=		0.00		Σ(rate ²)=		20661.19	
(Σrate) ² /10=		0.00		(Σrate) ² /10=		2066.12	
t-score = -1.0000							

DPM - Disintegrations per minute of tritiated (³H)-phosphate buffered saline.

RATE - Measured in 10⁻¹¹ Moles of tritiated (³H)-pbs per minute, by Specimen.

MEAN - Mean rate for each Group by Interval

SD - Standard deviation for each Group by Interval

SEM - Standard error of the mean for each Group by Interval

Rate², Σ(rate), Σ(rate²), (Σrate²) - data required for statistical analysis.

Paired, two-tailed Student T-Tests were applied to mean ³H-water rate data for Group I and Group II, at each time interval.

The parameters chosen for significance were selected as follows :

An α level = 0.05 ; Degrees of Freedom (df) = 18 ; t_α = 2.101

{H₀ : μ₁ ≠ μ₂} ; {H₁ : μ₁ = μ₂}

if calculated t-score < 2.101, then accept the null hypothesis

i.e. : "on the basis of the present study a difference could not be statistically guaranteed "

Table 2.2b. Tritiated water data arranged in Groups by Interval.

T ₁ =0.03 days	DPM	RATE	RATE ²	T=43 mins	DPM	RATE	RATE ²
115	118.42	2.82	7.95	116	4.61	0.11	0.01
106	21.90	0.52	0.27	101	69.58	1.66	2.76
107	61.83	1.47	2.16	102	121.81	2.90	8.41
179	239.94	5.71	32.60	172	234.59	5.59	31.25
152	72.14	1.72	2.96	203	41.03	0.98	0.96
148	33.61	0.80	0.64	156	133.00	3.17	10.05
181	33.33	0.79	0.62	133	193.01	4.60	21.16
244	146.88	3.50	12.25	235	3.12	0.07	0.00
243	23.52	0.56	0.31	249	48.90	1.16	1.35
241	279.24	6.65	44.22	253	130.92	3.12	9.73
mean	103.08	2.45		mean	98.06	2.34	
SD ±	92.61	2.21		SD ±	77.99	1.86	
SEM±	29.29	0.70		SEM±	24.66	0.59	
Σ(rate)=		24.54		Σ(rate)=		23.36	
Σ(rate ²)=		104.00		Σ(rate ²)=		85.68	
(Σrate) ² /10=		60.22		(Σrate) ² /10=		54.57	
t-score = 0.1294							
T ₂ =0.1 day	DPM	RATE	RATE ²	T=100	DPM	RATE	RATE ²
115	310.57	3.11	9.67	116	114.34	1.14	1.30
106	171.18	1.71	2.92	101	310.06	3.10	9.61
107	460.76	4.61	21.25	102	576.18	5.76	33.18
179	949.55	9.50	90.25	172	1074.70	10.75	115.56
152	304.10	3.04	9.24	203	252.95	2.53	6.40
148	420.71	4.21	17.72	156	420.57	4.21	17.72
181	206.75	2.07	4.28	133	322.03	3.22	10.37
244	665.40	6.65	44.22	235	279.90	2.80	7.84
243	106.30	1.06	1.12	249	168.10	1.68	2.82
241	625.72	6.26	39.19	253	495.71	4.96	24.60
mean	422.10	4.22		mean	401.45	4.02	
SD ±	261.57	2.62		SD ±	275.16	2.75	
SEM±	82.72	0.83		SEM±	87.01	0.87	
Σ(rate)=		42.22		Σ(rate)=		40.15	
Σ(rate ²)=		239.88		Σ(rate ²)=		229.41	
(Σrate) ² /10=		178.25		(Σrate) ² /10=		161.20	
t-score = 0.1723							

Table 2.2c. Tritiated water data arranged in Groups by Interval.

T ₃ =0.3 day	DPM	RATE	RATE ²	T=244	DPM	RATE	RATE ²
115	881.96	3.61	13.03	116	428.70	1.76	3.10
106	440.64	1.81	3.28	101	1081.42	4.43	19.62
107	3105.77	12.73	162.05	102	1316.71	5.40	29.16
179	1779.96	7.29	53.14	172	1836.02	7.52	56.55
152	1214.66	4.98	24.80	203	737.39	3.02	9.12
148	1273.20	5.22	27.25	156	1003.78	4.11	16.89
181	949.01	3.89	15.13	133	979.53	4.01	16.08
244	2195.84	9.00	81.00	235	1366.27	5.60	31.36
243	359.33	1.47	2.16	249	702.78	2.88	8.29
241	1934.61	7.93	62.88	253	1461.02	5.99	35.88
mean	1413.50	5.79		mean	1091.36	4.47	
SD ±	849.64	3.48		SD ±	415.43	1.70	
SEM±	268.68	1.10		SEM±	131.37	0.54	
Σ(rate)=	57.93			Σ(rate)=	44.72		
Σ(rate ²)=	444.73			Σ(rate ²)=	226.06		
(Σrate) ² /10=	335.59			(Σrate) ² /10=	199.99		
t-score = 1.0777							
T ₄ =1 day	DPM	RATE	RATE ²	T=820	DPM	RATE	RATE ²
115	833.24	1.02	1.04	116	462.19	0.56	0.31
106	520.50	0.63	0.40	101	1291.20	1.57	2.46
107	6456.21	7.87	61.94	102	3810.48	4.65	21.62
179	1009.68	1.23	1.51	172	964.26	1.18	1.39
152	1595.84	1.95	3.80	203	901.74	1.10	1.21
148	2744.88	3.35	11.22	156	3018.56	3.68	13.54
181	964.84	1.18	1.39	133	1329.76	1.62	2.62
244	2697.43	3.29	10.82	235	1866.85	2.28	5.20
243	592.85	0.72	0.52	249	1103.99	1.35	1.82
241	2393.25	2.92	8.53	253	2018.28	2.46	6.05
mean	1980.87	2.42		mean	1676.73	2.05	
SD ±	1788.73	2.18		SD ±	1037.44	1.27	
SEM±	565.65	0.69		SEM±	328.07	0.40	
Σ(rate)=	24.16			Σ(rate)=	20.45		
Σ(rate ²)=	101.17			Σ(rate ²)=	56.24		
(Σrate) ² /10=	58.37			(Σrate) ² /10=	41.82		
t-score = 0.4653							

Table 2.2d. Tritiated water data arranged in Groups by Interval.

T ₅ =3 days	DPM	RATE	RATE ²	T=2260	DPM	RATE	RATE ²
115	993.57	0.44	0.19	116	554.05	0.25	0.06
106	563.29	0.25	0.06	101	2185.77	0.97	0.94
107	5498.65	2.43	5.90	102	3758.52	1.66	2.76
179	961.73	0.43	0.18	172	951.83	0.42	0.18
152	1715.37	0.76	0.58	203	1085.82	0.48	0.23
148	3264.86	1.44	2.07	156	3229.09	1.43	2.04
181	1727.51	0.76	0.58	133	2324.26	1.03	1.06
244	3931.01	1.74	3.03	235	2215.75	0.98	0.96
243	836.83	0.37	0.14	249	1810.51	0.80	0.64
241	2021.27	0.89	0.79	253	2708.04	1.20	1.44
mean	2151.41	0.95		mean	2082.36	0.92	
SD ±	1598.74	0.71		SD ±	1015.64	0.45	
SEM±	505.57	0.22		SEM±	321.17	0.14	
Σ(rate)=		9.51		Σ(rate)=		9.22	
Σ(rate ²)=		13.53		Σ(rate ²)=		10.31	
(Σrate) ² /10=		9.04		(Σrate) ² /10=		8.50	
t-score = 0.1096							
T ₆ =10 days	DPM	RATE	RATE ²	T=8020	DPM	RATE	RATE ²
115	1502.55	0.19	0.04	116	1557.34	0.19	0.04
106	930.51	0.12	0.01	101	4553.85	0.57	0.32
107	7637.95	0.95	0.90	102	5716.09	0.71	0.50
179	1501.26	0.19	0.04	172	1406.76	0.18	0.03
152	2224.77	0.28	0.08	203	2304.99	0.29	0.08
148	4419.54	0.55	0.30	156	3231.96	0.40	0.16
181	2165.06	0.27	0.07	133	3121.44	0.39	0.15
244	5049.16	0.63	0.40	235	4280.37	0.53	0.28
243	1597.89	0.20	0.04	249	3372.37	0.42	0.18
241	2781.55	0.35	0.12	253	4788.30	0.60	0.36
mean	2981.02	0.37		mean	3433.35	0.43	
SD ±	2103.02	0.26		SD ±	1416.61	0.18	
SEM±	665.03	0.08		SEM±	447.97	0.06	
Σ(rate)=		3.73		Σ(rate)=		4.28	
Σ(rate ²)=		2.00		Σ(rate ²)=		2.11	
(Σrate) ² /10=		1.39		(Σrate) ² /10=		1.83	
t-score = -0.5530							

Table 2.2e. Tritiated water data arranged in Groups by Interval.

T ₇ =30 days	DPM	RATE	RATE ²	T=22420	DPM	RATE	RATE ²
115	6347.79	0.28	0.08	116	2779.08	0.12	0.01
106	3301.88	0.15	0.02	101	3638.50	0.16	0.03
107	9585.49	0.43	0.18	102	7005.04	0.31	0.10
179	3442.49	0.15	0.02	172	2157.75	0.10	0.01
152	2262.51	0.10	0.01	203	3083.57	0.14	0.02
148	7353.97	0.33	0.11	156	3395.98	0.15	0.02
181	2876.64	0.13	0.02	133	4023.45	0.18	0.03
244	8037.70	0.36	0.13	235	6150.04	0.27	0.07
243	3298.85	0.15	0.02	249	5437.25	0.24	0.06
241	5919.03	0.26	0.07	253	8292.15	0.37	0.14
mean	5242.64	0.23		mean	4596.28	0.20	
SD ±	2540.21	0.11		SD ±	2022.27	0.09	
SEM±	803.28	0.04		SEM±	639.50	0.03	
Σ(rate)=		2.34		Σ(rate)=		2.04	
Σ(rate ²)=		0.66		Σ(rate ²)=		0.49	
(Σrate) ² /10=		0.55		(Σrate) ² /10=		0.42	
t-score = 0.6563							
T ₈ =100 days	DPM	RATE	RATE ²	T=80020	DPM	RATE	RATE ²
115	10114.14	0.1264	0.0160	116	3764.53	0.0470	0.0022
106	5286.13	0.0661	0.0044	101	4116.84	0.0514	0.0026
107	10071.93	0.1259	0.0158	102	6945.85	0.0868	0.0075
179	5347.60	0.0668	0.0045	172	3127.31	0.0391	0.0015
152	3545.66	0.0443	0.0020	203	4165.82	0.0521	0.0062
148	8600.10	0.1075	0.0116	156	6302.66	0.0788	0.0034
181	5561.30	0.0695	0.0048	133	4634.91	0.0579	0.0034
244	10449.44	0.1306	0.0171	235	9581.57	0.1197	0.0143
243	4841.49	0.0605	0.0037	249	4643.87	0.0580	0.0034
241	7540.84	0.0942	0.0089	253	7555.50	0.0944	0.0089
mean	7135.86	0.0892		mean	5483.89	0.0685	
SD ±	2539.26	0.0317		SD ±	2039.40	0.0255	
SEM±	802.99	0.0100		SEM±	644.92	0.0081	
Σ(rate)=		0.8918		Σ(rate)=		0.0685	
Σ(rate ²)=		0.0886		Σ(rate) ² =		0.0528	
(Σrate) ² /10=		0.0795		(Σrate) ² /10=		0.0470	
t-score = 1.5704							

Table A2.2f Summary table two factor ANOVA - Lateral Internal Surface Area

Source	SS	df	MS	F
Total	57.92	19		
group I/II	0.69	1	0.69	1.34
LSA	26.02	1	26.02	13.34**
group/LSA	199.01	1	199.01	102.05**
error	31.22	16	1.95	

F = 8.53 for α level of 0.01
hence there is a significant difference attributable to the effect of lateral internal surface area.

Table A2.3a Peak Eugenol Concentration across Root Dentine - Smear present ($T_4 = 24$ hrs)

Group I	A	B	C	D	E	F	G	H
309	35.6	45.1	3015	1302	85.73	29.25	2.79×10^{-5}	9.53×10^{-6}
290	29.6	25.3	249	203	8.51	8.12	2.77×10^{-6}	2.64×10^{-6}
135	42.2	37.7	1782	387	42.74	10.40	1.39×10^{-5}	3.39×10^{-6}
282	56.5	46.9	1592	886	28.52	19.12	9.29×10^{-6}	6.23×10^{-6}
270	59.6	57.5	1813	1137	30.79	20.01	1.01×10^{-5}	6.52×10^{-6}
mean	44.70	42.50	1690	783	39.258	17.38	1.28×10^{-5}	5.67×10^{-6}
sem $\pm$	5.82	5.34	440	212	9.460	3.886	3.10×10^{-6}	1.24×10^{-3}

Table A2.3b Peak Eugenol Concentration across Root Dentine - Smear removed ($T_4 = 24$ hrs)

Group II	A	B	C	D	E	F	G	H
307	42.6	51.4	3727	1064	87.49	20.70	2.89×10^{-5}	6.83×10^{-6}
272	38.2	29.8	658	272	17.23	9.13	5.69×10^{-6}	3.01×10^{-6}
187	48.7	28.9	789	166	16.20	5.74	5.35×10^{-6}	1.89×10^{-6}
280	38.7	44.4	1421	923	36.72	20.79	1.21×10^{-5}	6.86×10^{-6}
263	55.1	56.0	1290	877	23.41	15.66	7.73×10^{-6}	5.17×10^{-4}
mean	44.66	42.10	1577	660	36.21	14.40	1.19×10^{-5}	4.76×10^{-6}
sem $\pm$	3.22	5.53	557	184	9.58	3.14	3.13×10^{-6}	1.01×10^{-6}

Table A2.3c Peak Eugenol Concentration across Root Dentine - Pooled Data

mean	44.68	42.30	1633	722	36.54	17.07	12.1×10^{-6}	5.22×10^{-6}
sem $\pm$	3.14	3.62	335	134	6.387	2.271	2.09×10^{-6}	0.75×10^{-6}

where : column A represents the wet dentine weight from the inner/pulpal sample (milligrams)
column B represents the wet dentine weight from the outer/periodontal sample(milligrams)
column C represents the raw dpm data from the inner/pulpal sample
column D represents the raw dpm data from the outer/periodontal sample
column E represents the eugenol concentration from the inner/pulpal sample (pmol/mg)
column F represents the eugenol concentration from the outer/periodontal sample (pmol/mg)
column G represents the eugenol Molality within the pulpal dentine layer
column H represents the eugenol Molality within the periodontal dentine layer

Table : A2.3d Peak Eugenol Concentration Gradient between pulp-periodontium (T₄ = 24 hours)

Group I - Smear present (Miltans)		Group II - Smear removed (EDTAC)	
Specimen No	Concentration gradient between pulp-periodontium	Specimen No	
309	2.32	307	
290	1.22	272	
135	4.61	187	
282	1.80	280	
270	1.59	263	
Mean concentration gradient across Root Dentine = (2.52 :1)			

Appendix 2.4

Activity Concentration of stock tritiated (^3H)-eugenol

A 1: 400 dilution of stock tritiated (^3H)-eugenol solution was prepared and its tritium activity determined by liquid scintillation counting (LSC), in Aquasol scintillant.

It was found that $2.5 \mu\text{L} \pm 0.01\%$ of parent tritiated (^3H)-eugenol stock represented 1.6×10^6 dpm at October 1987, with a tritium (^3H) counting efficiency of 59.5% and a $2\sigma\%$ error of $\pm 0.07\%$.

A 2.5×10^{-3} ml volume of stock is the equivalent of 16.3×10^{-6} moles when corrected for density and molecular weight.

Hence, 16.3×10^{-6} moles represents 1.6×10^6 d.p.m or, more significantly, that one (1) dpm = 10.2×10^{-12} moles of parent tritiated (^3H)-eugenol.

Therefore, since 1.6×10^6 d.p.m is the equivalent of 2.67×10^4 Bq or $0.721 \mu\text{Ci}$, as at October 1987, then the parent tritiated (^3H)-eugenol stock solution had a Tritium Activity concentration (A_t) = $164.65 \times 10^7 \text{ Bq.mole}^{-1}$ ($445 \text{ mCi.mole}^{-1}$).

Appendix 2.5 Pilot study : Partitioning Co-efficient and Relative Quenching Capacities of Chloroform versus Xylene, for Tritium (^3H) in Aqueous solution.

Pilot work was undertaken in two parts to determine the partitioning co-efficient of xylene relative to chloroform in pbs and their relative quenching capacities on standard concentrations of tritiated (^3H)-eugenol.

In Part 1, a standard solution of tritiated (^3H)-pbs was prepared from 25 mCurie/ml tritiated (^3H)-water by diluting 5 mL $\pm 0.5\%$ of isotope in 62.5 ml $\pm 0.1\%$ (w/v) of pbs. This represented a 1:1000 dilution and an actual tritium (^3H) Activity Concentration equivalent to 1 mCurie/ml of pbs, (i.e. 3.7×10^4 Bq/ml or 2.22×10^6 dpm/ml).

Using similar methodology to the main study, 5.0 ml $\pm 0.5\%$ volumes of tritiated (^3H)-pbs were added to 5.0 ml $\pm 0.1\%$ of each solvent. This produced a heterogeneous mixture with solvent supernatant and an aqueous precipitate. Samples of each phase were then analysed by LSC to determine their partitioning co-efficients, i.e., how successfully each solvent was able to exclude aqueous isotope from the unlabelled solvent phase (solvent miscibility in water)

The isotope/solvent mixture was vigorously shaken for 20 seconds and allowed to settle for at least 5 minutes. The agitation process was repeated three times and a 1.0 ± 0.05 ml aliquot drawn off from each of the respective phases. These samples were added to 10 ml $\pm 0.1\%$ of Aquasol scintillant and analysed by LSC as previously described (vid: 4.3.1). Four (4) samples were examined with the following results :

Table A2.5.a Solvent Partitioning Co-Efficient (W/S)

Chloroform (1:10 ³)			Xylene (1:10 ⁴)		
		† c.e.			† c.e.
S_c	718 dpm	21%	S_x	111 dpm	67%
W_c	2.54×10^6 dpm	45%	W_x	2.61×10^6 dpm	46%

† c.e. = tritium counting efficiency

In Part 2 of this work, three (3) standard solutions of tritiated (^3H)-eugenol in pbs, were prepared and analysed in the same manner as above, to compare the cpm data for each solvent and then to derive their relative quenching capacity. The concentrations of these solutions were :

- A. 1:100 tritiated (^3H)-eugenol in pbs
- B. 1:2000 tritiated (^3H)-eugenol in pbs
- C. 1:8000 tritiated (^3H)-eugenol in pbs

Five (5) samples were examined for each dilution ; one for each phase (n=2) and solvent (n=2), and one for each parent pbs dilution without solvent extraction (n=1) :

Table A2.5.b. Relative Quenching Capacity.

A. 1:100 tritiated (^3H)-eugenol in pbs					
Chloroform			Xylene		
		t.c.e.			t.c.e.
S_c	92.5×10^4 cpm	28%	S_x	244.1×10^4 cpm	61%
W_c	32.8×10^4 cpm	35%	W_x	48.2×10^4 cpm	35%
pbs	245.3×10^4 cpm	36%			
B. 1:2000 tritiated (^3H)-eugenol in pbs					
S_c	44.3×10^3 cpm	27%	S_x	113.5×10^3 cpm	59%
W_c	15.5×10^3 cpm	37%	W_x	18.9×10^3 cpm	35%
pbs	129.6×10^3 cpm	35%			
C. 1:8000 tritiated (^3H)-eugenol in pbs					
S_c	16.2×10^3 cpm	29%	S_x	35.1×10^3 cpm	60%
W_c	4.4×10^3 cpm	37%	W_x	6.0×10^3 cpm	37%
pbs	36.4×10^3 cpm	37%			

† c.e. = tritium counting efficiency

The relevant conclusions drawn from these results were that :

1. Xylene was an efficient partitioning agent, excluding 99.999% of tritiated (^3H)-pbs from the solvent phase in Part 1.
2. Xylene exhibited approximately 2-5 times less quenching potential than chloroform, with respect to aqueous solutions of tritiated (^3H)-eugenol, in Aquasol scintillant, in Part 2.

Xylene was therefore selected as the solvent for extraction of eugenol from the collected eluant sample pbs solutions in preference to the previously advocated chloroform technique of Becker et al (1983) and Hume (1984b) - vid: 4.3.1.

Appendix 3.1 Reference Chemical Data

Eugenol

Eugenol is known systematically as 2-Methoxy-4-(2-propenyl)phenol, but is more often referred to as either 4-Allyl-2-methoxyphenol or 4-Allylguaiacol. It is a simple alkene substituted phenol.

Eugenol is an "Essential Oil" which is commonly extracted from Oil of Cloves.

It represents 85-90% (v/v) of the latter and is considered to be its principal therapeutic moiety.

Empirical formula - $C_{10}H_{12}O_2$

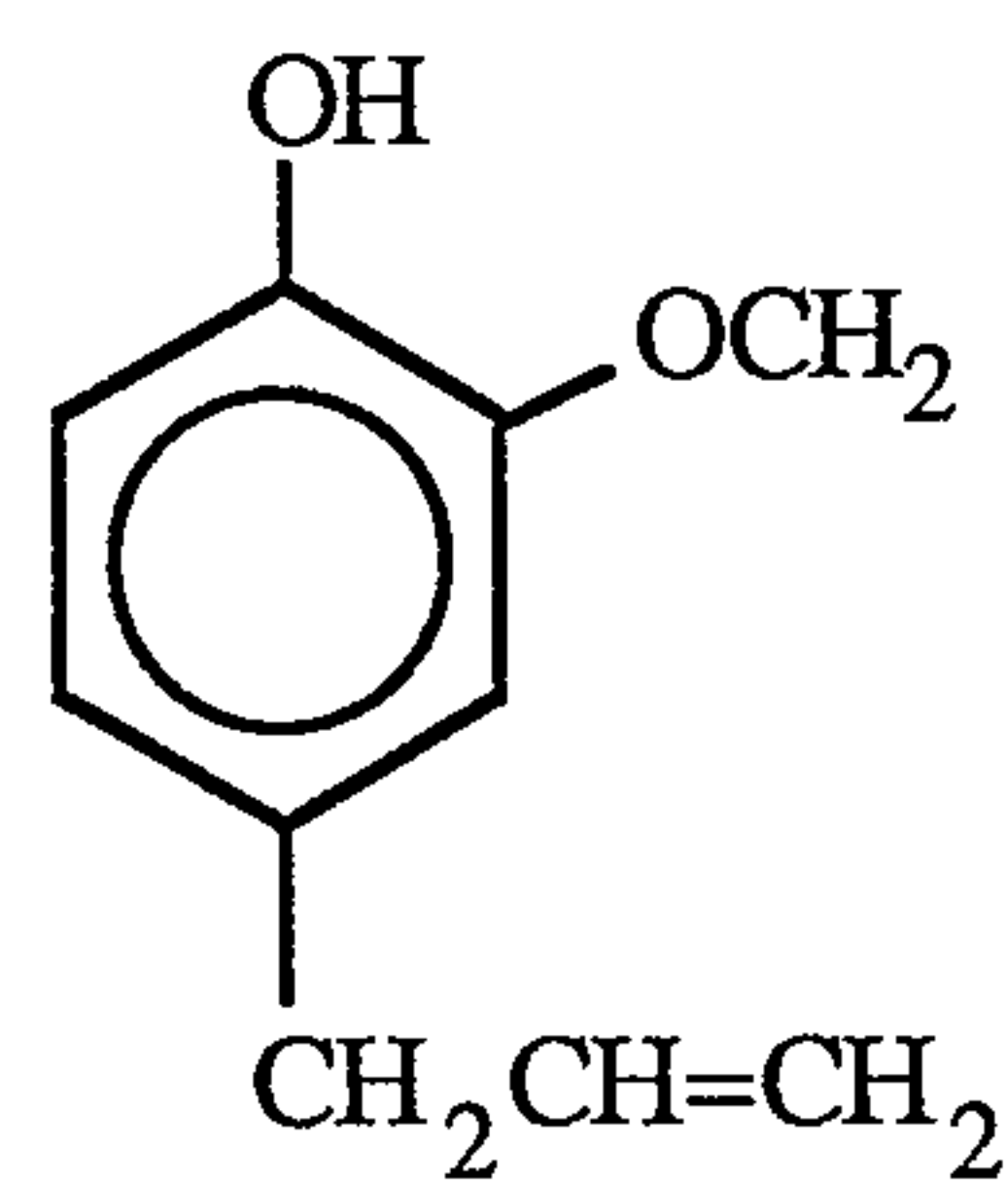
Molecular weight = 164.2,

Density (g/ml) = 1.0664 at 20 °C.

Insoluble in aqueous solution above $10^{-3}M$;

miscible with alcohol, diethyl ether, benzene, chloroform ;

and soluble in acetic acid or alkali solutions.



Appendix 3.2 Units of Radioactivity

1 microCurie (μCi) = 2.22×10^6 disintegrations.min⁻¹ (dpm)
 = 3.7×10^4 disintegrations.sec⁻¹ (dps)
 = 3.7×10^4 Becquerels (Bq)

1 Becquerel (Bq) = 60 disintegrations per minute (dpm)

NET-262 User supplied substance labelled (by DuPont Industries - USA)
 with tritium (³H) via catalytic proton exchange

Tritium (³H) half life = 12.43 years

Appendix 4.1 STATISTICAL FORMULA

Student T-Test

Paired two-tailed Student t-Test calculations were based on the following formula from Phillips, D.S., (1978) p.82

$$t \text{ score} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\sum(X_1)^2 - (\sum X_1)^2/N_1 + \sum(X_2)^2 - (\sum X_2)^2/N_2}{N_1 + N_2 - 2}} \times \left[\frac{1}{N_1} + \frac{1}{N_2} \right]}$$

where : $\bar{X}_1$ = mean of Miltons sample group

$\bar{X}_2$ = mean of EDTAC sample group

H_0 : $\bar{X}_1 \neq \bar{X}_2$

H_1 : $\bar{X}_1 = \bar{X}_2$

α - Level = 0.05, (two-tailed analysis), $t = 2.101$

number of statistics in each sample group (N) = 10

degrees of freedom (df), i.e. $(N_1 + N_2 - 2) = 18$

D.R. : "there is no difference between the means of Group I and II."

4.2 MATHEMATICAL FORMULA

Volume of an Elliptical Frustum

Volume of an elliptical frustum (V_e) = one third the height multiplied by the sum of, the areas of the frustum bases and the square root of their products.

$$V_e = \frac{1}{3} h (B_1 + B_2 + \sqrt{B_1 \cdot B_2})$$

i.e., $= \frac{1}{3} h [\pi (r^2 + ab) + \sqrt{\pi (r^2 \cdot ab)}]$

Lateral Surface Area of an Elliptical Frustum

Lateral area of an Elliptical frustum (S_e) = half the sum of the perimeters of each of the bases multiplied by the height of slant of the frustum.

$$S_e = \frac{1}{2} (P_1 + P_2) s$$

i.e., $= \frac{1}{2} \{2\pi \cdot [r + \sqrt{\frac{1}{2}(a^2 + b^2)}]\} \times \sqrt{(a-r)^2 + h^2}$

where B_1 and B_2 are the areas of the bases of the frustum,
 P_1 and P_2 are the perimeters of the bases of the frustum,
 s = the height of slant of the frustum,
 a = major axis of the coronal ellipse,
 b = minor axis of the coronal ellipse,
 r = radius of the apical seat (half the M.A.F. size),
 h = length (altitude) of canal available for diffusion.

adapted from Beyers, W. H. (1981) ; CRC Standard Mathematical Tables ; 26th Ed.; CRC Press, Florida; pp124-129.

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